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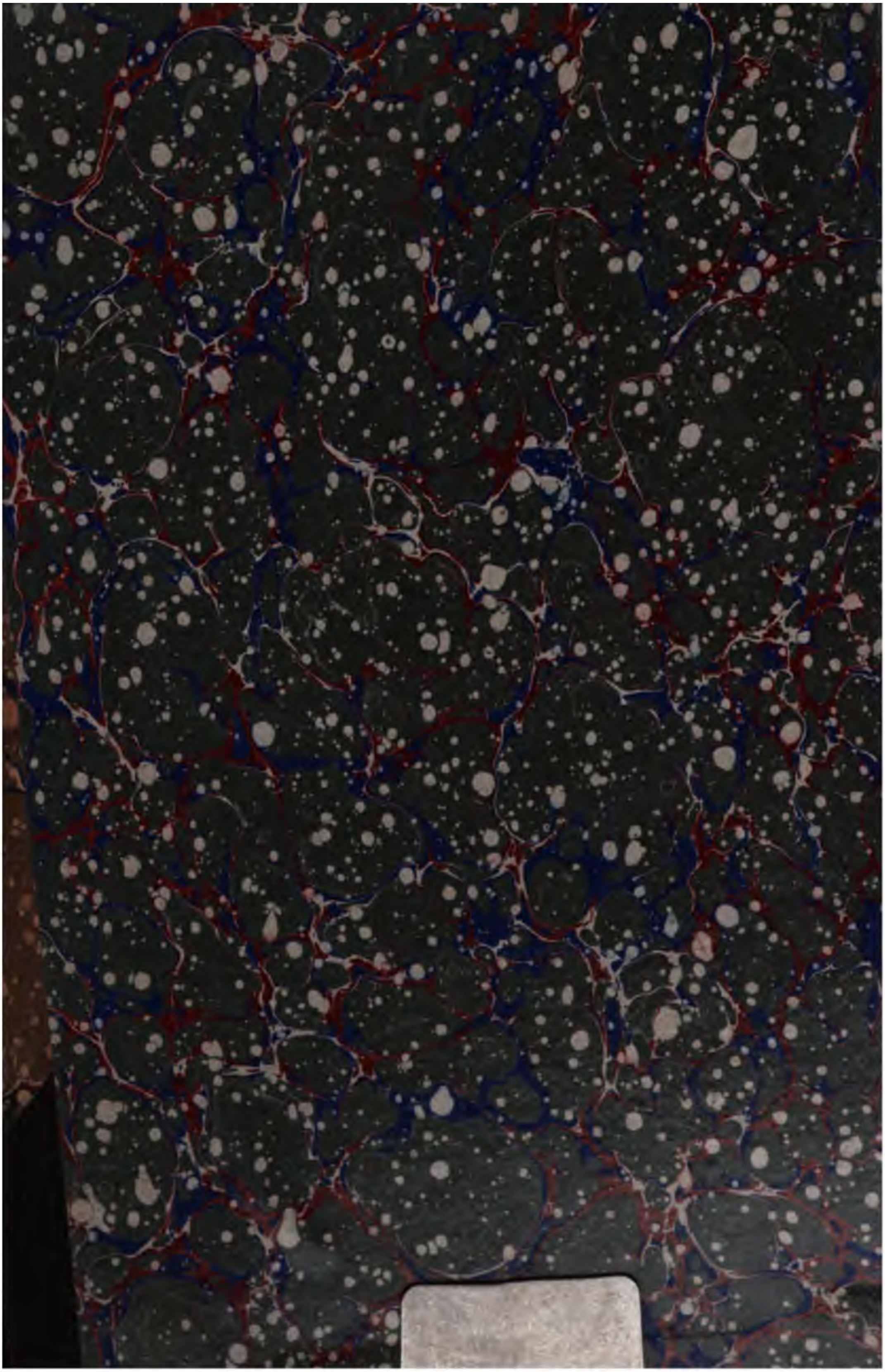

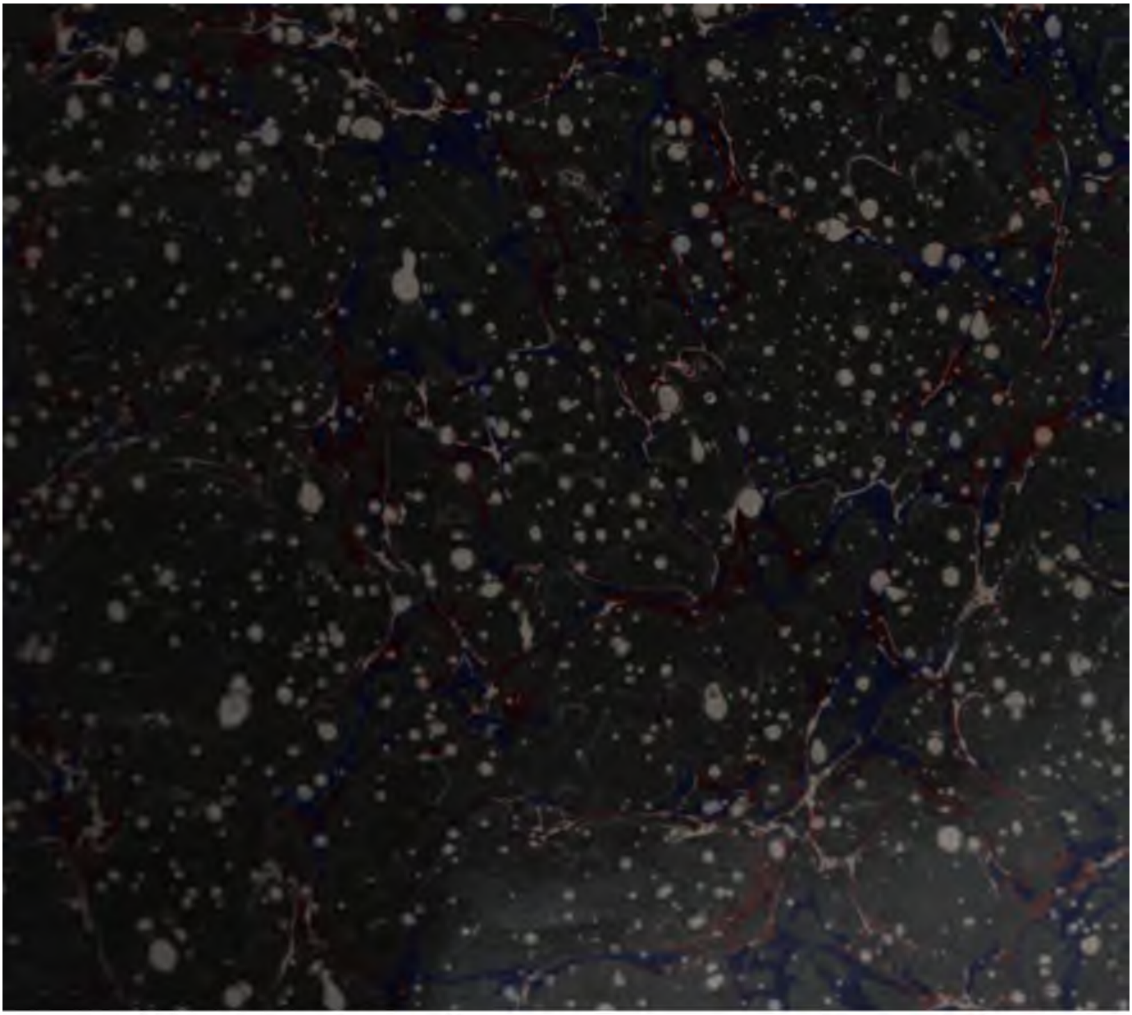

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ERRATUM.

Page 176, fourth line from bottom: for '*Lygodesmus*' read '*Zygodesmus*.'

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MILES JOSEPH BERKELEY.

BORN 1803. DIED 1889.

(*With Portrait*).

THE task is never an easy one for those of one generation in science to express in a few words the precise nature of the debt which they owe to their predecessors. The effect of personal influence is always somewhat intangible and necessarily fades with time. The final verdict of the scientific historian cannot be reached except in a more remote perspective. Perhaps in detail it never can be reached satisfactorily at all, owing to the difficulty of interpreting rightly the ideas of one period in terms of those of another.

In the case of Berkeley the task seems tolerably clear. In the first place he was the virtual founder of British Mycology. His labours in this field began in 1836, when he undertook for Sir William Hooker the description of the British species for that author's British Flora. He became from this time the leading authority in Mycology, at least as far as the British Empire was concerned. He is believed to have published in all descriptions of some 6,000 species, and this enormous labour he accomplished with a skill and precision which leaves little room for doubt as to its durability.

Ten years later his long study of Mycology led him by a transition—less obvious then, but which seems natural to us now—to the study of the pathology of plants. He was the first perhaps, to treat the subject in a systematic manner, and though the long series of papers which he devoted to it remain buried in the periodicals in which they appeared, they did their work in establishing once for all its treatment on a scientific basis. His memoir on the Potato-Murrain, published in 1846, would even half a century later be thought a model for similar researches, and has not been added to in any material particular.

Another decade, and in 1857 he published his Introduction to Cryptogamic Botany, a memorable book which may still be consulted with advantage and always with pleasure. At the time and for long after it played somewhat the part filled in later years by Sachs' Lehrbuch. It was the first comprehensive treatise of the kind in any language. While summing up the striking results of foreign research, it was no mere compilation but everywhere drew materials from personal observation. The facts it contains still reveal themselves from time to time with unexpected freshness. One of the most striking instances is the account of the curious organism *Emericella*, in which Berkeley came very near to an anticipation of Schwendener's hypothesis of the nature of Lichens. The great merit of all his work is the true biological spirit with which it was pervaded. Perhaps Mycology in this respect has the advantage over other branches of taxonomy in that. for the most part. it requires its material to be dealt with in the living state.

The facts of personal history required to complete this brief appreciation are few. A man of good family, he was educated at Rugby and at Christ's College, Cambridge. As was the case with Darwin, he derived his scientific impulse in great measure from Henslow. The first objects of his study were Mollusca. and he always preserved the spirit of the naturalist. He became a clerk in holy orders in 1827, but never enjoyed any considerable preferment. Though his life was one of indefatigable labour, he was at no time rewarded by more than a moderate competence. His appearance till within a few years of his death, when overtaken by infirmity, was one of splendid distinction. To this the portrait now given scarcely does justice: but it is unfortunately the only one which is available.

W. T. THISELTON-DYER.

The Structure and Development of Grinnellia americana, Harv.

BY

MELVIN A. BRANNON.

With Plates I-IV.

THE investigations on the reproduction and development of *Grinnellia americana* were undertaken for the reasons that *Grinnellia* is distinctively an American species and that little was known of the structure and development of the cystocarp The work was taken up at the suggestion of Dr William A. Setchell, Director of the botanical department of the Marine Biological Laboratory at Woods Hole, Mass., and was brought to its present stage in the summer of 1894. For Dr. Setchell's continuous interest and very valuable assistance in the prosecution of the work I am under deep obligation. Through the kindness of the Trustees and of Dr William Trelease, Director of the Missouri Botanical Gardens, I occupied the room subscribed for by them during the summers I was engaged in the study of *Grinnellia*; for this privilege I am also greatly indebted.

Grinnellia americana, as stated above, is distinctively an American Alga, never, so far as learned, having been reported from other waters than those of the west Atlantic coast. It ranges from the mouth of the Weymouth River, Mass., to

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Norfolk, New Jersey, and appears abundantly in Long Island and Vineyard Sounds. The writer has also collected fine specimens at Nantucket. Possessed of brilliant lake-red colour and delicate texture, it constitutes a notable specimen in every representative collection of the New England marine flora.

HISTORY.

The elder Agardh placed this Alga in the genus *Delesseria*, which 'then comprehended almost every Alga with a red membranaceous leaf-like frond, and also included within its limits *Plocamium* and *Stenogramme*.' It was referred later to *Nitophyllum*, but was found to differ radically in the form of conceptacle, shape of frond, and position of midrib. For a time it seemed that it was to have fellowship with *Hemineura*, but the different position of conceptacle, lack of similarity in nervation and ramification, gave sufficient distinction to deny admission to this genus.

After a somewhat careful study of the plant, Dr. Harvey raised it to the rank of an independent genus. He named it *Grinnellia*, as a memorial to the 'noble conduct of Henry Grinnell of New York, chief promoter of the search after the missing Arctic expedition of Sir John Franklin.'

GENERAL MORPHOLOGY.

In his description of *Grinnellia*, Harvey says that this Alga has a frond which is 'rosy-red, leaf-like, delicately membranaceous, areolated, symmetrical. traversed by a slender percurrent midrib. Conceptacles scattered over the surface of the membrane, bottle-shaped, with a prominent orifice; placenta basal, somewhat prominent, crowned with a pulvinate tuft of subdichotomous spore-threads whose terminal cells are earliest ripened. Spores elliptic oblong or roundish. Tetraspores tripartite, immersed in scattered shapeless cellular warts.'

In addition to these statements regarding the gross anatomy of *Grinnellia*, it should be said that in well-developed plants lateral veins arise from the basal region of the percurrent midrib. In frayed fronds these lateral veins occasionally develop into strong branches, simulating the action sometimes occurring at the tip of the frond after an injury to the apex; viz. bifurcation of the midrib in its subsequent growth. Often these proliferations are the only vestiges of the frayed frond; and thus is demonstrated the fact that clusters of young plants can arise vegetatively by compounding the remnants of the old one. In this respect *Grinnellia* resembles closely the associated genus *Delesseria*.

The leaf-like frond is supported by a short somewhat cylindrical stalk which terminates in a dense, disk-shaped holdfast. As Mr. M. C. Potter suggests in his study of the thallus of *Delesseria*, this differentiation of parts in the frond of *Grinnellia* gives the single plant a resemblance in structure and functions to a dicotyledonous leaf. The foliar expansion serves as an organ of assimilation; the midrib, veins, and stalk furnish channels for conducting food-material and reservoirs for its storage. This last function is evidenced by the initial growth of numerous proliferations from the midrib and stalk of frayed fronds.

Both asexual and sexual reproduction prevail in *Grinnellia americana*, and, unlike many of the Florideae, monoecism is never present. The two methods of reproduction and the differentiation of the sexes give certain peculiar characteristics to the vegetative structure of the different fronds.

Male plants are usually much smaller, less abundant, and have more delicate fronds than either female or asexual individuals. The largest dimensions of those collected at Woods Hole were 7 cm. wide and 15 cm. long. The fronds have a smooth surface and, if superficially examined, would be considered sterile; but a careful inspection reveals numerous lighter coloured portions occupying from two to many times the area of the normal cells. Sections through these areas show them to be patches of antheridia producing large numbers of antherozoids.

The female plants average larger than the tetrasporic. Their cystocarps are arranged promiscuously on either side of the frond, while occasional sections through cystocarps growing base to base were obtained. The cystocarps are cone-shaped, with carpostomes in the crown of each affording a place through which the mature carpospores may escape. Within the fully-developed conceptacle, dichotomous chains of lake-red, slightly elliptical carpospores are readily seen by focussing through the pericarp; but the carpospores are so numerous, so densely massed together, and of such deep colour in the mature cystocarps, that one can determine very little of the true structure of this organ by means of optical sections. As in the non-sexual plants, the fruiting-bodies in the male and female frond are less mature near the margin and apex.

On certain cystocarpic plants numerous proliferations are developed on both sides of the frond. These plants are apparently healthy and normal in all other respects. The proliferations may become greatly developed and give the plant an unusual appearance, very similar to that of leaves bearing many small galls. These outgrowths are composed usually of from two to three axial rows of cells corresponding to those of the frond, except that their contents are clear and granular. These cells are surrounded by one or two layers of smaller cells, having the characteristic lake-red colour of the frond (Fig. 14). An unusually fine opportunity for the study of the origin and development of the cystocarp is offered by these proliferations (Figs. 3, 14, 17). They were critically examined in whole and sectioned preparations, in stages from the earliest formation of the procarp to the mature cystocarp. The cystocarps thus developed often appeared pedicellate on account of the length of the proliferation, in the outer end of which they were borne (Fig. 3). No tetrasporic or antheridial plants were found bearing these proliferations.

The asexual plants outnumbered the female even during July and August, the period when the latter reached their maximum number at Woods Hole. In shape, colour, and texture of frond the mature tetrasporic plants are very similar to the cystocarpic, but the decided contrast between the rough, shapeless, tetrasporic incrassations and the smooth conical cystocarps, easily enables one to distinguish them unaided by the microscope. Each incrassation contains from four to forty tripartite tetraspores, which closely resemble the carpospores.

HABITAT.

Grinnellia has been described by Dr. Harvey as an Alga growing 'on stones and shells in four or five fathoms of water.' In the collections of *Grinnellia* made at Woods Hole and Nantucket it was found growing abundantly on the piles of wharves, at a depth of one to one and a half fathoms below high tide-mark. After severe storms it frequently drifts ashore in company with many other Algae which are torn from the slightly submerged rocks and shells.

Along the Massachusetts coast it thrives best in quiet waters, though fine specimens are seen on rocks and shells submerged one to two fathoms in the 'holes' connecting adjoining bays and indentations, through which the tidal currents run at a speed of eight to ten miles an hour. Mr. Isaac Holden has kindly allowed me to quote from his observations of this Alga made at Bridgeport, Conn., where it attains very considerable dimensions, his largest mounted specimens giving the royal measurements of 25 cm. wide by 65 cm. long. He says he has found it growing in strong tide-currents, but that it acquires its most luxuriant growth in quiet waters, thus corroborating the observations made along the Massachusetts coast. He has collected it from just below low water-mark to the depth of six fathoms, and has secured many fine specimens from the bottom, when wading at low tide, bringing with them the stones or shells, particularly oyster-shells, to which they were attached. Early in August adult plants are very apt to become detached and float in large numbers. From this last observation it appears that the mature plants afford their spores a rapid and wholesale method of distribution.

GENERAL HISTOLOGY.

The entire surface of the plant is covered with a thick gelatinous secretion, which causes it to adhere firmly to paper when mounted.

The vegetative cells, forming a single layer in the foliar expansion of the frond, are polygonal in shape, have thick cell-walls, protoplasm peripherally arranged containing granular material, a large nucleus, and a great number of red chromatophores (Fig. 1 *f*). These chromatophores are also found in the superficial cells of the midrib, the stalk and the upper surface of the holdfast. The cells in the apical and marginal (merismatic) portions of the frond are smaller, have more delicate cell-walls, and are far more active than those in the middle and basal regions of the frond.

The midrib is three to five layers of cells in thickness. The central cells are very large, elongated, and angular. The superficial cells are much smaller and more circular than those in the centre. All of them have a thick cell-wall and a thin peripheral layer of protoplasm containing a large amount of granular material (Fig. 1 *m*).

Transverse sections of the dense disk-shaped holdfast show that it is composed of a central region of large oval and oblong cells surrounded by layers of cells which gradually diminish in size and increase in numbers toward the surface of the organ, where they are quite uniformly roundish and form a strong cortical region (Fig. 2). Surrounding the entire holdfast is a very thick yellowish coat of cellulose, through which project many small rhizoidal filaments. These filaments are composed of one to many rows of thin-walled cells, and serve to absorb food-material as well as to fix the plant more firmly to its substratum. The cells of the holdfasts are completely filled with protoplasm and granular material; have a gelatinous, cellulose cell-wall, and are connected with one another by protoplasmic pits.

Sections through the holdfast, midrib, and frond show protoplasmic pits connecting adjoining joint-cells. It is not possible to demonstrate that open communication for interchange of nuclei, chromatophores, and other protoplasmic products between adjacent vegetative cells is established by these pits. In Schmitz's discussion of them he says they are traversed by plasma-cords which serve for conduction of dynamic influences from cell to cell. He thinks that a transfer of dissolved food-material from cell to cell is facilitated because of the pores in the separating membrane of the pit, but that migration of protoplasm by means of these open communications is inadmissible. He further states that open communication between cells of Algae is rare, and that when formed, as in Corallineae, the pores result from the subsequent modification of the common dissepiment of adjacent thallus-cells.

His views regarding the pits between thallus-cells of Algae are evidently correct with reference to the organic connexion of the cells of *Grinnellia*, with one exception—the connexion between the procarpic cells. As will be seen in the discussion of the procarp, the procarpic cells are connected, not by pits, but by open pores, which are established, possibly, in the manner ascribed by him to Corallineae.

PHYSIOLOGY.

Plants were found growing on the outer surface of the outermost piles of wharves at Nantucket and Woods Hole, having an eastern, southern, and western exposure to sunlight; while very few plants were found on the exposed surface of the north piles. Whether this was due to lack of light can hardly be stated, as other conditions, decidedly unfavourable to growth, were present. Not a single plant was found on the inside of outer piles nor on the outside of piles in any great degree shaded, while several other Algae belonging to the groups of the Chlorophyceae and Rhodophyceae were discovered growing in these places. This indicates that *Grinnellia* is far more dependent upon light than many of the Algae associated with it.

While direct sunlight appears necessary for the continued growth of the plant, it cannot endure strong light when only slightly submerged. If after collection the plants are placed in water in a shallow vessel, exposed to the direct rays of the sun, their normal colour rapidly changes to a brownish yellow, and they give every evidence of rapid decay, evidently due to the effect of direct sunlight and the accompanying rise of temperature. Direct experiments were not made to determine the sensitiveness of *Grinnellia* to changes of temperature alone, but incidentally it was observed that a sudden variation of 10° to 15° C. from normal (temperature of 15° C. in the water of Vineyard Sound) for any length of time is destructive to the tissue. Fishermen reported collections of this plant in the winter. Having cut through the ice they could scrape it from the piles, in water of which the temperature was probably not lower than 2° to 5° C., while in the summer the temperature of these waters, as previously stated, is 15° to 17° C.

The three types of fronds, antheridial, carposporic, and tetrasporic, show a similar degree of sensitiveness to direct light and sudden rise of temperature, but can be preserved in a normal condition for several days in the laboratory if properly shaded, if the normal temperature of the containing water be maintained, and if the sea-water be changed every thirty-six to forty-eight hours.

Nowhere are the effects produced by the change of light and temperature more perfectly shown than in the process of sporing and germination. The spores are liberated in great numbers during the night, particularly the second night after the plants are collected. However, numerous experiments proved that this process occurs in some degree during the day, differing in this respect from the sporing methods observed in some other Florideae, notably *Champia parvula*.

Immediately after collection, the carposporic and tetrasporic plants were separated in order to study the germination of their respective spores. It was found that the carpospores are usually closely grouped in great numbers immediately beneath the cystocarps from which they come, while the tetraspores are promiscuously distributed in much less abundance over the bottom of the dish. A gelatinous secretion begins to form about both carpospores and tetraspores very soon after they pass into the water, which serves to protect, to hold the spores together, and to fix them to the bottom of the dish or other substratum. The fact that they immediately fall to the bottom of the containing vessel shows that they have a greater specific gravity than the frond, which is probably due to the fact that their chromoplasts and other contents are denser than those of the cells of the parent frond.

In a few hours after the liberation of the carpospores they are so thoroughly fixed to the substratum by the gelatinous secretion, that it is necessary to subject them to the full force of a strong stream of water in order to loosen them. So far as could be determined by experiment, they become much more firmly attached than the tetraspores, which, in every case, are found to be easily detached from their position before germination. These phenomena lead to the belief that the special function of the asexual spores of Algae is identical with that of the asexual spores of Fungi; viz. immediate distribution. The carpospores differ so little in colour and shape from the tetraspores, both being lake-red and elliptic-oblong that they are scarcely distinguishable from one another. They agree closely in size averaging 30 μ by 36 μ, just preceding liberation. Shortly afterward they become more spherical and enlarge to 36 μ by 48 μ. On account of the densely aggregated chromoplasts in the newly-deposited spores, it is extremely difficult to distinguish the nucleus. However, when treated with five per cent. acetic, or one per cent. picric acid for ten minutes, washed thoroughly in distilled water, and then stained with Delafield's haematoxylin, the nucleus of each spore is readily recognized.

GERMINATION OF SPORES.

The carpospores and tetraspores having been separated in the manner above mentioned, experiments in germination were undertaken without possibility of confusing the respective action.

Because of Protozoa and minute Crustaceans, which greedily devoured the growing spores, great difficulty was experienced in the observation of their development. Immunity from these creatures was obtained by using water which had been kept for an hour at a temperature of 95° C., so as to secure a high temperature with but little evaporation, and thus to prevent a larger percentage of salt than is present in normal sea-water. Had water containing an excess of salt been used, it would have proved, according to Oltmann's experiments, destructive to germination.

After the water had cooled sufficiently, it was slowly filtered through a sand and cotton filter, an operation which secured a twofold object—removal of foreign bodies, and thorough aeration. Following these methods, the germination of carpospores and tetraspores gave very satisfactory results.

In the beginning of the process of germination, the gelatinous envelope, which upon application of chlor-iodide of zinc gives a cellulose-reaction, is secreted, and is arranged in two to three concentric layers about the carpospores (Fig. 5 *b, c, d*); while the single layer which surrounds the tetraspores is comparatively thin and homogeneous. With subsequent imbition of water the spores swell and become lighter-coloured, the form also changing from elliptical to egg- or oval-shape (Figs. 4 *a, b*; 5 *b, c, d*). Cell-division first makes itself manifest by transverse fission at the apical end, from twenty-four to thirty-six hours after the spores are deposited, a process which is usually repeated at corresponding intervals, giving rise to a filament of from three to five cells. While this filamentous growth is taking place at the apical end, simultaneous or closely succeeding development begins at the basal portion of the spore (Fig. 6 *b, b'*); and after the formation of three to six transverse walls, lateral branches develop, forming the holdfasts of the young plant (Fig. 7 *h*). These are usually only a few cells in length, irregular in outline and incurved at the tip, enabling them to fix the young plant firmly to its substratum. Because of the nature of its holdfasts, the young fronds are often found growing in epiphytic fashion on older *Grinnellia*-plants and other Algae.

At this period, the rapid growth in the middle of the filament is characterized by the appearance of vacuoles and the rearrangement of chromatophores in such definitely transverse and radiating lines that the appearance of cell-division is produced. As the spore continues developing, these lines of chromatophores are arranged lengthwise of the cells (Fig. 7 *c*), vacuoles appear and the colour of the contents becomes much lighter and almost disappears in the outer terminal cells of the filament (Fig. 7 *v*).

Cell-division in the germinating spore is limited to one plane until the formation of a frond proper begins, when fission takes place in two planes along the lines of the collected chromatophores; and subsequently, with the development of the midrib, occurs in three planes in the median region of the frond.

For frequent observation of the progress of germination, pieces of mica were placed beneath the fronds until sufficiently covered with spores. The mica was then transferred to shallow dishes of water, and was examined often during the early stages of spore development. This recurring disturbance proved injurious to the spores, and they seldom survived the treatment longer than a week. When unmolested and supplied with fresh water daily, they continued to grow for a period of two to three weeks.

To determine the varying effect of different rays of light upon their development, dishes containing spores were covered with colourless, red and blue glass respectively. These receptacles were then protected from direct sunlight, and supplied with the boiled sea-water which was changed every thirty-six to forty-eight hours. The spores under the colourless glass gave much better results than those in open dishes, living from ten to thirteen days, which was due, perhaps, to the exclusion of dust and the maintenance of a lower temperature. The average of results of experiments with carpospores and tetraspores shows that they live for a longer time, attain greater size, and possess a more vivid colour when kept under blue glass than when covered with red, and in both instances give more satisfactory results than those germinating under the colourless glass. These conclusions, while not final, give additional weight to the belief that this plant is particularly sensitive to light.

Under the most favourable conditions (beneath blue glass), the spores developed into young fronds of twenty-four cells; and on adult fronds collected, young plants of exactly comparable development were found growing in epiphytic fashion.

With the addition of these and more advanced forms, it was possible to pass in review the successive phases from the unicellular spores to the fully-developed membranaceous fronds.

Not only does *Grinnellia* reproduce itself by means of spores, but also vegetatively, in two ways. First, by the method referred to above where proliferations arise from the remnants of frayed fronds. Very many of these specimens were collected in the latter part of the season from piles which had been scraped earlier in the summer, at which time only fragmentary fronds of *Grinnellia* had been left attached. The second method of vegetative reproduction was observed in small portions of the frond which had been severed accidentally from the parent plant. These fragments, containing a short portion of the midrib, attached themselves by their cut ends to the bottom of the porcelain dishes, and grew vigorously as long as they were supplied with the usual favourable conditions.

DEVELOPMENT OF ANTHERIDIA.

In his work on the fructification of the Florideae, Dr. Schmitz states that, in all cases examined, the reproductive organs originate and develop by apical growth : 'An diesem Thallus entstehen die Sexualzellen durch Differenzirung einzelner Endzellen des ganzen Systems verzweigter Zellfäden.'

Grinnellia americana seemed an exception to Schmitz's law of development, because of the patches of antheridia and the cone-shaped conceptacles which originate from the membranaceous male and female fronds. Dr. Schmitz does not mention this peculiar Alga, which was probably inaccessible to him. To prove, therefore, whether this genus is an apparent or a real exception to his law, made the study of its development exceedingly interesting.

In the development of the antheridia, individual vegetative cells in different parts of the frond are observed to change from a pronounced red to a lighter colour, possibly due to an accompanying development of a large amount of granular matter of a highly refractive character. Similar changes are observed to follow immediately in many of the cells adjoining these centres, causing the male fronds to be patched promiscuously on both sides with numerous collections of these lighter-coloured granular cells. These individual groups occupy irregular areas varying from two to twenty times the surface of the average vegetative cell, and are covered with a thicker gelatinous layer than the vegetative portion of the frond (Fig. 9). Cross-sections of these regions show that the significance of the excessive granular substance in this instance is the modification of vegetative tissue in preparation for the formation of reproductive elements. The sections were prepared from antheridial fronds which had been stained two hours in Delafield's haematoxylin. They show that the modified vegetative cells divide transversely, and that each of the daughter-cells repeats the process at both ends, in a plane at right angles to the first plane of division thus giving rise to three layers of cells instead of one (Fig. 8).

The protoplasm of the cells last formed collects into a dense mass at the distal end and forms a spherical body, which separates by a constriction of the cell-wall below it. (Fig. 8 *d, d′ c c′*.) The spherical cell thus formed is an antherozoid.

Presently these small spherical bodies separate from their mother-cells and pass into the gelatinous layer which covers the patches of antheridia.

These antherozoids are non-motile, consequently depending upon water-currents for distribution so as to come into contact with trichogynes. Repeated tests for their nuclei gave negative results, though the granular contents show deeply-stained fragmentary particles.

Because the male plants are smaller and apparently much more rare than the female, and produce non-ciliated antherozoids, it is obvious that the male plants must generate an extraordinarily large number of them. This demand is abundantly supplied by the myriads of antherozoids developed in the apical manner described above.

DEVELOPMENT OF THE CYSTOCARP.

The method of the development of the cystocarps, which are promiscuously distributed on either side of the female frond of *Grinnellia*, appears more difficult to harmonize with the theory of Schmitz than does the manner of formation of the frond and the antheridia. The study of the initial development of the cystocarp, however, not only shows that it conforms to his theory of the development of most of the Florideae, but further testifies that the whole tissue of this leaf-like Alga is developed by apical growth of ramifying threads. In the apical and marginal regions of partially mature female plants, and distributed promiscuously over the entire surface of young female fronds, are isolated individual cells assuming a triangular shape (Fig. 10). They are directed apically outward and upward in such a manner that if a line were drawn through the long axis of any one of them to the median plane of the frond, an angle of 50° to 75° with the midrib would be formed. These are centres of active growth, and develop groups of cells (Fig. 11 and Fig. 12) similar in appearance and outline to those at the apices of the growing plants. After twenty to thirty cells have been formed in the plane of the frond, growth begins in the third plane, producing a papilla-like thickening in these portions (Fig. 13).

The procarp consists of three cells and arises as a lateral branch from one of the large joint-cells in the plane of the frond (Fig. 14). In the haematoxylin preparations of mature procarpic cells it is found that each contains a well-defined nucleus within the densely granular, protoplasmic contents, and that these cells are connected by open pores which perforate their delicate cell-walls. The granular material passing through these pores gives a beaded, strand-like appearance to the protoplasmic connexion between adjacent procarpic cells. The basal procarpic cell is frequently the smallest in the branch and is connected with the supporting cell by a pore, and not by a pit as are the four contiguous thallus-cells which, with the supporting cell, form a characteristic group in the floor of the young cystocarp.

It is possible that the early connexions between the cells of the procarpic branch were pits which became open pores by the absorption of the delicate cell-wall at the points of communication.

The uppermost cell of the procarpic chain becomes the carpogonium from which the trichogyne develops (Fig. 14 *tr*). The trichogyne is subject to considerable variation. In some instances it is branched and much elongated (Fig. 17 *b*); in others it is simple, twisted, and of moderate length (Fig. 15). The granular protoplasmic contents of the trichogyne are in marked contrast to the clear gelatinous sheath enveloping it. Just above the carpogonium, a knee-shaped enlargement frequently occurs (Figs. 14 and 17 *k*). At this point the longitudinal axis of the trichogyne changes its direction and makes an angle of 30° to 40° with its former axis. This enlargement on one side of the basal region may be the cause of the changed direction of growth in the trichogyne, or may be an accompanying phenomenon due to the resistance which the young trichogyne must overcome when pressing between the cells of the rapidly-forming pericarp.

In Schmitz's discussion of the origin of the female sexual cells of the Florideae, he states that without exception they develop from the end-cells of shorter or longer side-branches of the whole system of ramification of thallus-filaments; that these branches are formed sometimes as secondary side-branches subsequently to the formation of other ramifications; and that, in all cases, the female sexual cell, the carpogonium, is formed from the end-cell of the side-branch by permitting a process to project from its apex which develops into a more or less long hair-like trichogyne.

As has been shown in the foregoing description of the origin of the procarpium and carpogonium, *Grinnellia* agrees with many of the Florideae in the development of these organs.

It was impossible to discover antherozoids fusing with the trichogynes of freshly collected material, therefore several young female fronds were placed in a dish of water and covered with antheridial plants. The same degree of care was observed with respect to the change of filtered sea-water at stated periods and the excess of light and heat as had been found necessary in the successful germination of spores and the preservation of living *Grinnellia* plants. Very satisfactory results followed, and several cases of fusion and partial fusion were found in sections of specimens artificially brought together.

The carpogonium is fertilized by the fusion of one or more antherozoids with the trichogyne (Fig. 19 *a*). It was impossible to identify the nucleus in the antherozoids, and equally difficult to determine whether the trichogyne possessed a nucleus. Hence no union of nuclei was observed when the cell-contents of the antherozoid were intimately associated with those of the trichogyne. In the latter there were individual and grouped particles, which took a deeper stain when treated with Delafield's haematoxylin than the surrounding non-granular substance.

In somewhat older specimens the trichogyne is separated from the fertilized carpogonium by a cellulose plug which is formed in the neck of the trichogyne. Shortly after this, zoogloea-bacteria collect about the distal end of the trichogyne and cause its rapid disintegration (Fig. 14 *b*). This shows the evanescent character of the organ, which decomposes and falls away in a short time after receiving the contents of the fusing antherozoid.

As there is no indication of a conjugating tube connecting the carpogonium to the large auxiliary cell which supports the procarpic branch, and as there are open pores connecting the cells of the procarpium, it seems evident that, with the substitution of pores for pits, Bornet's explanation of the method of propagating the fertilizing influence from the carpogonium to the cell which develops the sporiferous tissue must be accepted in this Alga.

Very soon after the fertilizing contents of the carpogonium have been transmitted to the auxiliary cell (the large thallus-cell which supports the procarpic branch), it begins an active growth. It increases in size, develops a very thick cellulose cell-wall, and becomes gorged with dense, yellowish, protoplasmic contents. A similar and accompanying phenomenon is observed to occur in the four large auxiliary cells which immediately surround this supporting cell of the procarp and are found in the plane of the frond (Fig. 16).

These cells are nucleated, have the same general position with reference to one another (Fig. 25), and form a distinctive group in all young cystocarps.

In their study of *Gracilaria*, Bornet and Thuret refer to a group of cells in the basal region of the fruiting body which resemble this group in *Grinnellia*. In a subsequent investigation of the procarpium and fruit of *Gracilaria*, Mr. Johnson mentions the same cells and considers them a part of the procarp. In *Grinnellia* they are auxiliary cells of which the central one develops the sporiferous tissue subsequently to receiving the fertilizing influence of the carpogonium.

The developmental changes in the young cystocarp are very rapid from this period. The central cell of this group, as noted, is the centre of growth of the sporiferous filaments, and the four adjacent cells receive and supply food-material for the development of the sporiferous tissue. In all preparations of a certain stage it was observed that the central cell gave rise to an upper and a lower daughter-cell (Fig. 20 *b*, *c*). These cells contained the same dense, yellowish, protoplasmic contents which characterized the cell from which they came. The daughter-cells, resulting from the division of the upper cell (Fig. 26 *d*) multiply rapidly and soon form a papilla (*pa*) of four to six cells extending from the floor obliquely into the cavity of the cystocarp. The plane of cleavage in the cells of the papilla is not regular but angular and concave on the upper surface of older, and convex on the lower surface of younger cells (Fig. 18). Marginal cells are cut off from the surface of these and give rise to most of the sporiferous filaments (Fig. 23). All of these cells are connected by pits and contain the same dense, yellowish, protoplasmic contents which characterized the auxiliary cells.

Sporiferous filaments continue to originate at different times, so that in a median longitudinal section of a partially mature cystocarp fully developed spores may be found on one side, and half mature to very young ones on the other (Fig. 22).

The sporiferous filaments have a somewhat unique, though uniform, method of development. They usually grow in the form of a central chain of cells, from each unit of which a whorl of branches is developed consisting of two to three cells (Fig. 24). Primarily, the cells of each branch are connected by long protoplasmic filaments (Fig. 18 *pi*), but the cells gradually enlarge until this neck-like connexion is so abbreviated that they are almost in contact, showing merely a simple protoplasmic pit in the very short connexion between their walls (Fig. 23 *p*). These enlarged cells become densely filled with protoplasm and give rise to the dichotomously branching chains of carpospores. Usually three to five carpospores originate from each sporiferous branch by repeated abstriction of its terminal portion (Fig. 23 *c*). At first the carpospores are small, irregular in form (Fig. 26 *c*), and possess a clear granular appearance. Presently they enlarge (Fig. 26 *cp*), passing through an elongated oblong-elliptical form (Fig. 26 *c'*) to a true oval or spherical shape (Fig. 26 *m*). As the spores gradually increase in size, they acquire a reddish colour which finally becomes a lake-red, the characteristic colour of all mature spores of *Grinnellia.* This process is repeated by every one of the sporiferous filaments developing from the large central basal cell, in this way giving rise to a very large number of carpospores, averaging from 300 to 400, in every mature cystocarp. The stimulation resulting from fertilization of the young cystocarp is manifest not only in the origin and active growth of the sporiferous filaments, but also in the rapid development of other portions, such as the pericarp, basal cortical tissue and sterile filaments (Fig. 21).

From the moment the joint-cell at the base of the procarpic branch begins to enlarge and acquire dense protoplasmic contents, the growth of the pericarp is accelerated. The cells on the upper surface of the cystocarp multiply rapidly and become elevated by the growth of tissue in the fruiting portion of the young cystocarp. As this surface-tissue, which is to form the pericarp, further develops, it is apparent that its irregular arrangement of cells is becoming regular, and that a number of filaments, growing apically, are united with each other by lateral branches. These pericarpic filaments not only branch in the surface-plane of the pericarp, but also at right angles to that plane, forming two- to three-celled branches which are connected with the terminal cells of the sterile filaments within the cystocarpic cavity (Fig. 18 *sp*). The branches from the pericarpic filaments decrease as the filaments grow outward and upward until their apices are in contact and form a circle about an open portion over the central region of the cystocarpic cavity. These filaments now change the direction of growth, which had previously formed an angle of 60° to 75° with the surface-plane of the frond, so that they grow almost at right angles with that surface (Fig. 22 *cr*). The apical cells divide four to six times and growth discontinues. Thus a cone-shaped wall is developed about the cystocarp, and a carpostome, very rarely two, is formed in the apex of the cystocarp by the circularly-arranged terminal cells of the pericarpic filaments.

The lower cortical tissue, composed of a few cells at the time of the fertilization of the procarp, begins to increase immediately after fertilization and forms a supporting tissue of four to six layers of cells between the floor or basal cystocarpic cells (consisting of the five auxiliary and the surrounding cells lying in the horizontal plane of the frond) and the pericarp, enclosing the lower portion of the cystocarp (Fig. 22 *c*). These cortical cells have thin walls and clear granular contents, indicating that their function is, at least partially, transmission of food to the mother-cells of the sporiferous filaments.

The body-cavity enlarges, *pari passu*, with the development of numerous sporiferous and sterile filaments, the latter connecting the cortical tissue at the base of the cystocarp with various portions of the rapidly developing pericarp.

These sterile filaments consist of a few, long, narrow cells having an appearance similar to that of the cortical cells (Figs. 18, 26 *sf*). The function of these filaments is somewhat problematical. The fact that they originate from the basal cortical region of the cystocarp, which is in immediate contact with the group of dense, protoplasmic, auxiliary cells, from one of whose daughter-cells the sporiferous tissue develops, and that they are joined to the pericarpial wall, strongly suggests that their function is to conduct nutritive material from the pericarp through the cortical tissue to the mother-cells of the sporiferous filaments. It seems hardly probable that they afford a support to the pericarp, as it is composed of two to three layers of cells arranged in the manner of an arch about the body-cavity of the cystocarp (Fig. 21 *pr*).

The fact that the cells given off in whorls from the central cells of the sporiferous filaments are small with clear sparsely granular contents at first, in subsequent development becoming greatly enlarged and crowded with dense granular substance, gives additional testimony to the belief that the sterile filaments connecting pericarp and cortical cells in the base of the cystocarp (Fig. 21) are agents for conducting food-material from the pericarp to the egg-cells of the cystocarp.

In Mr. T. Johnson's work on *Sphaerococcus* and *Gracilaria*, he figures and describes a complicated fusion of the procarpic cells shortly after fertilization of the trichogyne. A very large cell is the result of this fusion of several cells and from this the ooblastema-filaments develop. Nothing of this nature is discovered in the growth of the cystocarp of *Grinnellia*. It seems, on the contrary, that the large protoplasmic companion-cells simply contribute nourishment to the large central cell through the pits in the walls of their connecting processes (Fig. 26 *p*), and that this large cell gives rise to a few ooblastema-filaments and to a papilla-like growth of cells containing dense protoplasmic contents from which nearly all the ooblastema-filaments develop (Figs. 24 25). In this way the production of these filaments is continued at different intervals and gives rise to chains of carpospores of various ages, ranging from mature to very young forms (Figs. 22, 26).

The lack of fusion in the auxiliary cells of *Grinnellia* is one of the most notable points observed in the study of its development. It is especially peculiar, as fusion of basal, procarpic cells has been reported in the related genus *Gracilaria*. A careful examination of a very large number of stained and unstained preparations of median longitudinal and transverse sections of young and old cystocarps failed to reveal a single case of cell-fusion The cell-walls of the old, empty, auxiliary cells in the base of the mature cystocarps were unabsorbed, though often irregular and distorted, because the cell-contents had been used in supplying food-material to the ooblastema-filaments and carpospores. As only a thin membrane is left to mark the cell-walls of the empty auxiliary cells, the freezing method was especially valuable in exactly determining whether there had been any true fusion of adjacent cells Any process employed in preparation for sectioning, which would have produced even a slight rupture in the frail walls of these old exhausted cells, would almost certainly have given preparations showing the empty cells connected by wide openings, thus leading to the conclusion that cell-fusion between these auxiliary cells had taken place.

During much of the time devoted to the initial study of *Grinnellia*, many attempts were made to carry it through paraffine for the purpose of sectioning, but none were successful. It was found on examination from time to time, as the plants were being prepared for imbedding, that they endured the action of absolute alcohol and the oils very well; but in every case the tissue was found to be too delicate to endure the temperature necessary in the final process of imbedding, even when the softest paraffine, melting at the low temperature of 45° C., was employed.

After repeated failures with paraffine the celloidin method was tried. This proved somewhat more satisfactory, though the prolonged treatment of sections with the oils used in dissolving celloidin shrivelled and distorted them to such an extent as to render observations partial and uncertain in their results. Finally, having secured an abundance of favourable material, and adopting the freezing methods and apparatus perfected by Mr. W. J. V. Osterhout of Brown University, the successful study of the cystocarpic development of *Grinnellia* was undertaken, and very gratifying results obtained.

The great advantages of the freezing method in work on *Grinnellia* are appreciated in the ability to make rapid preparation of fresh tissue and to secure a great number of sections with comparatively little labour. This latter advantage makes the process especially valuable in showing the relation of the evanescent trichogyne to the procarp. It is difficult to observe this relationship by other methods, since many of the cystocarps may be too young or too old by a few days to show clearly the early stages of procarpic development.

A brief summary of this method may be given as follows:—

A portion of the fresh frond is arranged in several layers, held firmly together and placed in dilute gum-arabic on the freezing chamber. In a few minutes it is frozen sufficiently for sectioning.

The sections in this work were cut ·03 mm. in thickness, and, mounted in dilute glycerine, were ready for examination.

In some cases sections were stained with Delafield's haematoxylin in order to bring out distinctly the thin gelatinous coat which surrounds the procarpic cells and, in more advanced stages of the cystocarp, the thick cell-walls of the auxiliary cells and ooblastema-filaments.

To summarize the chief points developed in the study of *Grinnellia americana*:—

1. *Grinnellia americana* is distinctively an American marine Alga. It was erected into an independent genus by Dr. Harvey.
2. There are no distinctive differences in the vegetative structure of the male, female, and tetrasporic fronds.
3. This Alga flourishes most luxuriantly in quiet waters. In such conditions the fronds separate from their holdfasts late in the summer; and, rising to the surface, are carried away by the shore-currents, thus effecting a wide and wholesale distribution of the fruiting bodies.
4. The cells, vegetative and reproductive, are nucleated, surrounded by thick cell-walls and, with one exception, connected with one another by protoplasmic pits. In the case of the exception, the cells of the procarp, they are connected by open pores.
5. Adult plants are very sensitive to intense light and increasing temperature; but, on the other hand, will not grow in shady places.
6. Mutilated plants proliferate readily, and thus a single frayed frond may give rise to a large number of vegetatively-produced plants. Not only do these proliferations develop when injured, but the female plants frequently bear proliferations, in the terminal end of which may be found a cystocarp, which appears pedicellate.

Plants may originate vegetatively by regeneration of the frond from small portions which have been cut from the parent plant.

7. It is found by experiment that the carpospores and tetraspores are excellently adapted to the study of the various phenomena attending germination, for while they respond readily to change of intensity of light, temperature, and salinity of the surrounding water, yet they are sufficiently hardy to develop into young plants when artificially supplied with favourable conditions.

8. The non-motile antherozoids are developed in enormous numbers by the abstriction of the terminal portion of the apical cells of the antheridia.

9. The cystocarp begins to develop by the modification and apical growth of a joint-thallus-cell.

10. The procarp, consisting of three cells, is developed from the supporting thallus-cell in the base of the young cystocarp. Its apical cell becomes the carpogonium. Subsequently, the fertilized contents of the carpogonium are transferred through the open pores connecting the procarpic cells to the supporting thallus-cell which becomes the central one of the five auxiliary cells.

11. The trichogynes are often branched, and as many as five simple ones may grow from a cystocarp borne on a proliferation.

12. Fusion of the antherozoid with the trichogyne results in great stimulation to the thallus-cell at the base of the procarp, and rapid disintegration of the trichogyne, which is a very evanescent organ.

13. The sporiferous filaments are developed as chains of central cells, from each of which a whorl of spore-producing threads may originate.

14. The carpospores arise acropetally from the branches of the sporiferous filaments, and not interstitially.

15. The development of the cystocarps of *Grinnellia* agrees with that reported for *Gracilaria* in that the body-cavity develops schizogenetically, and that the sterile filaments connect the pericarp with the cortical tissue below the group

of highly refractive, yellow cells at the base of the procarpium. It differs in that the pericarp is only two to three layers of cells in thickness, and that no fusion of cells is observed in the basal cystocarpic region of *Grinnellia.* As previously stated, this is one of the most salient points developed in the study of *Grinnellia.* This subject was more interesting, and received more special attention, because fusion of cells in the basal cystocarpic region had been reported for other Florideae, notably the closely related genus *Gracilaria.*

16. It is perfectly evident that fusion of cells in the basal region of the developing cystocarp does not occur; but that the large amount of nourishment supplied to the ooblastema-filaments by the cells growing directly from the joint-cell at the base of the cystocarp, is received directly through the pits from the group of densely protoplasmic auxiliary cells in the floor of the fruiting body, and indirectly, by transmission from the pericarp through the sterile filaments.

17. *Grinnellia americana* conforms in every phase of development to Schmitz's law of apical growth. This law is exemplified in the germination of the spores, the growth of the fronds, the origin and development of antheridia and cystocarps, and the final separation of antherozoids and carpospores.

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EXPLANATION OF FIGURES IN PLATES I-IV.

Illustrating Mr. Brannon's paper on *Grinnellia americana*, Harv.

Fig. 1. Transverse section of a frond. *m*, showing the region of the midrib. *f*, the region of the frond which is only one layer of cells in thickness. × 125.

Fig. 2. Transverse section of the holdfast. *c*, thick, yellowish cuticle. *co*, the cortical portion of the holdfast. *r*, one of the rhizoidal filaments. × 725.

Fig. 3. *c*, cystocarps borne on the distal end of the proliferations. × 30.

Fig. 4. Two carpospores two days old showing a thick, cellulose cell-wall. *a*, spherical spore before germination, and *b*, the spore having become elliptical in outline shortly after the formation of an apical cell. × 500.

Fig. 5. Three germinating carpospores showing gelatinous coats of cellulose arranged concentrically. *b*, two-celled stage of germination. *c* and *d*, more advanced and showing apical growth. × 500.

Fig. 6. Carpospore four days old. *b*, *a*, a^1 are the cells which are to produce the foliar portion, and b^1 the cell which is to develop the holdfast portion of the plant. × 500.

Fig. 7. A tetraspore eight days after beginning to germinate. *h*, a filamentous holdfast. *c*, the longitudinally arranged chromatophores, and *v*, the vacuoles. × 500.

Fig. 8. Transverse section of a cluster of antheridia. *d*, a mother-spermatium-cell with the protoplasm collected in its distal end. d^1, showing the separation of the young antherozoid. *c*, more advanced, and c^1 a young antherozoid with shining granular contents completely separated from mother-cell. (Haematoxylin preparation.) × 1200.

Fig. 9. Surface-view of a patch of young antheridia (*an*). × 325.

Fig. 10. Portion of the marginal region of a young female frond showing a three-celled cystocarp which is developing apically from a modified vegetative cell. × 220.

Figs. 11 and 12 show more advanced stages of Fig. 10. × 220.

Fig. 13. A surface-view of a young cystocarp showing the trichogyne (*t*) and the apical cell (*a*) corresponding to *a* of Figs. 11 and 12. x 220.

Fig. 14. Transverse section of a young cystocarp borne on a proliferation. *tr*, trichogyne. *k*, the knee-like projection at the base of the trichogyne. *b*, collection of Bacteria about the disintegrating apex of the trichogyne. *a*, the carpogonium. *po*, the open pore connecting the fertilized carpogonium with its hypogynal cell. *d*, the joint-thallus-cell which gives rise to the procarpium. *c*, cortical tissue in the pericarpic region of the young cystocarp. x 440.

Fig. 15. Transverse section of a young cystocarp showing the joint-thallus-cell (*b*) which gives rise to the procarpic branch. *tr*, twisted trichogyne. *a*, carpogonium. *au* and *au'*, two auxiliary cells. x 440.

Fig. 16. The group of five auxiliary cells which characterizes every young cystocarp. *p*, pit-like connexions. *a*, the thallus-cell which gives rise to the procarpic branch and, subsequently to fertilization of the carpogonium, receives its fertilized contents. *nu*, nucleus which appears distinctly in these cells. *d*, daughter-cell from the central auxiliary cell (*a*). x 725.

Fig. 17. Transverse section of a proliferation which bears a young cystocarp having two trichogynes. *b*, a branched trichogyne. *a*, carpogonium. *k*, the knee-like growth on the trichogyne *tr*. x 800.

Fig. 18. Portion of a transverse section of a young cystocarp. *a*, central auxiliary cell. 1, 2, and 3, companion-auxiliary cells. *d*, daughter-cell producing an ooblastema-filament and a papilla of densely protoplasmic cells from which most of the ooblastema-filaments develop. The cleavage plane in these cells of the papilla is very irregular. *pi*, protoplasmic pits connecting adjacent sporiferous cells. *sf*, sterile filaments connecting the basal cortical portion of the cystocarp to the inner branch-cells (*sp*) of the pericarp (*pr*). x 725.

Fig. 19. An optical section of a proliferation which has a young cystocarp with two trichogynes (Haematoxylin preparation). *a* and a^1, two antherozoids in contact with the trichogyne. *b*, a strongly stained body in the protoplasmic contents of the trichogyne. x 440.

Fig. 20. Transverse section of a young cystocarp in the plane *mn* of Fig. 16. *a*, the joint-thallus-cell which bears the procarpium and receives the fertilized contents of its carpogonium. *c*, an upper, and *b*, a lower daughter cell of the mother-cell (*a*); *c* has developed ooblastema-filaments *sf*. 1 and 2 are companion auxiliary cells. x 575.

Fig. 21. Obliquely transverse section of a young cystocarp. *a*, central auxiliary cell. *d*, the upper daughter-cell from the mother-cell (*a*). *pa*, the obliquely developed papilla of cells from which the ooblastema filaments develop. *pi*, protoplasmic pit. *sf*, sterile filaments connecting the basal cortical region (*c*) of the cystocarp with its pericarp (*pr*). x 800.

Fig. 22. Transverse section of a partially mature cystocarp showing chains of mature carpospores (*sp*) on one side and half mature (*sp'*) on the other side. *sf*, sterile filaments connecting the basal cortical tissue to the double-walled pericarp. *cr*, carpostome formed by the surrounding terminal cells of the pericarpial filaments. x 135.

Fig. 23. Portion of the transverse section of the papillary cells with marginal cells (*m*) from which sporiferous filaments develop. *c*, young carpospore abstricting acropetally. *p*, protoplasmic pit. x 440.

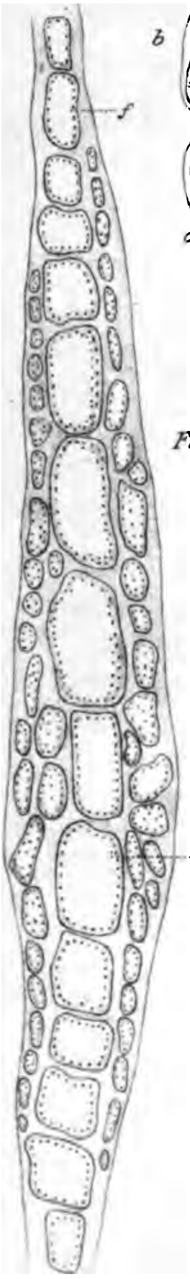
Fig. 24. Oblique section through the basal region of the cystocarp. *a*, central

auxiliary cell with companion-auxiliary cells (1, 3, and 2). *d*, upper daughter-cell bearing the ooblastemà-filaments (*sp*) which develop whorls of sporiferous filaments from central cells in the chain. *pi*, protoplasmic pits. *sf*, sterile filament which develops from one of the auxiliary cells surrounding the central one of the auxiliary group. × 725.

Fig. 25. A somewhat later stage of the auxiliary cells than that shown in Fig. 16. *of*, ooblastema-filament. *pa*, papilla from which other ooblastema-filaments are to develop. × 725.

Fig. 26. Transverse section of a semi-mature cystocarp. *sp*, dichotomous sporiferous filaments which are abstricting young carpospores (*c*) from their outer ends. *cp*, elliptical half mature carpospores. *m*, mature carpospores which were separated from their parent sporiferous filaments and remained in the cystocarpic cavity of the sectioned body. *d*, portion of the upper daughter-cell from the central auxiliary cell (*a*). *pa*, papilla of cells which developed from the daughter-cell (*d*). *sf*, sterile filaments, and *p*, protoplasmic pits. × 725.

All figures were drawn by the author. The third figure was drawn from an herbarium specimen. All the others were drawn with the aid of the camera lucida from preparations of fresh material.



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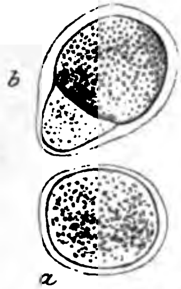

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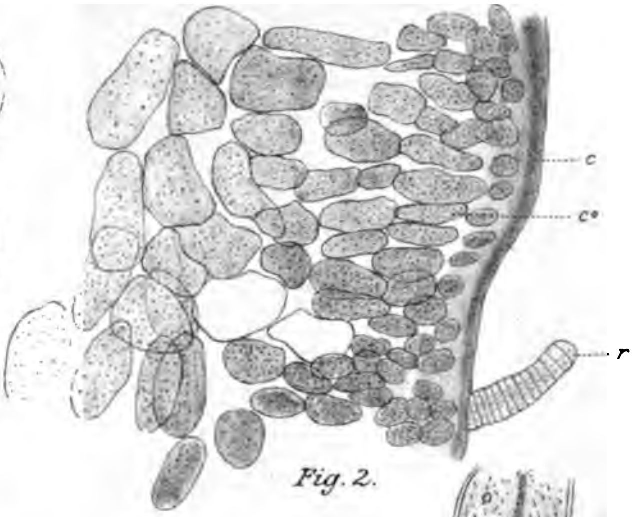

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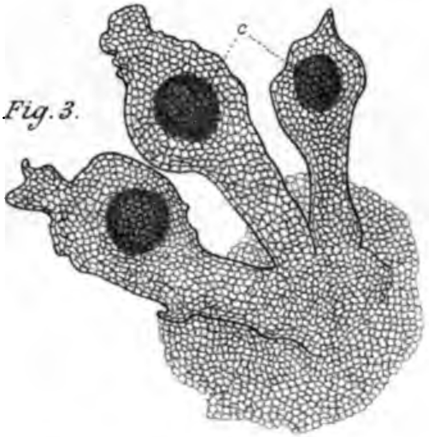

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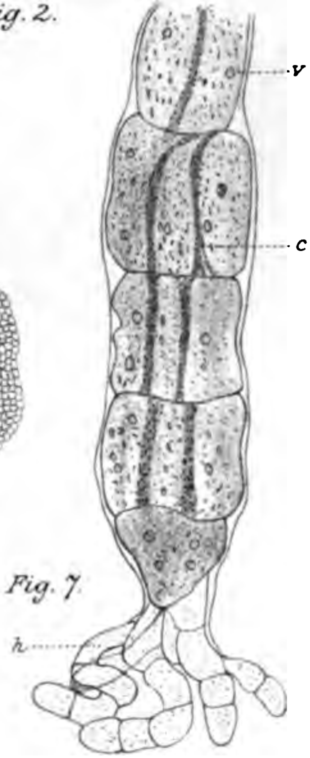

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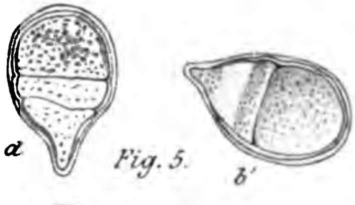

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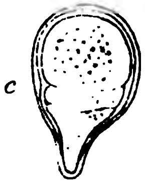

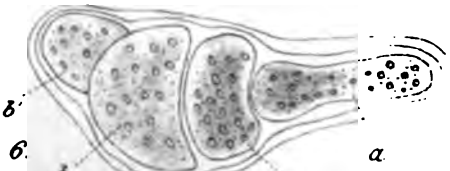

Fig. 6.

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Fig. 8.

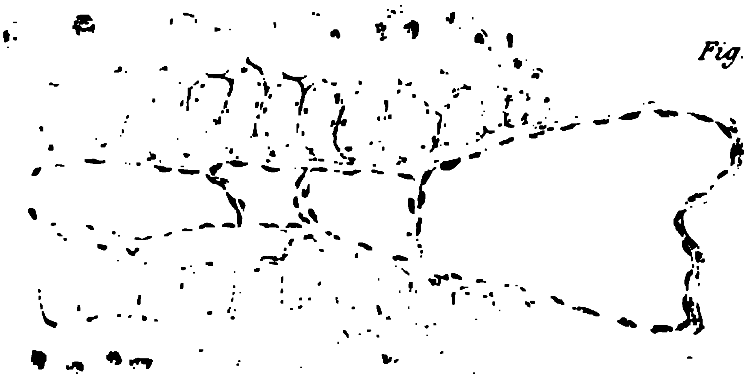
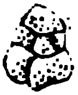
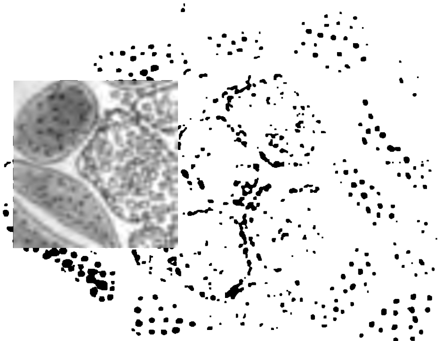


Fig 10

Fig 11





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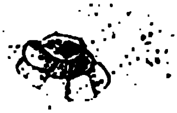

Fig 14

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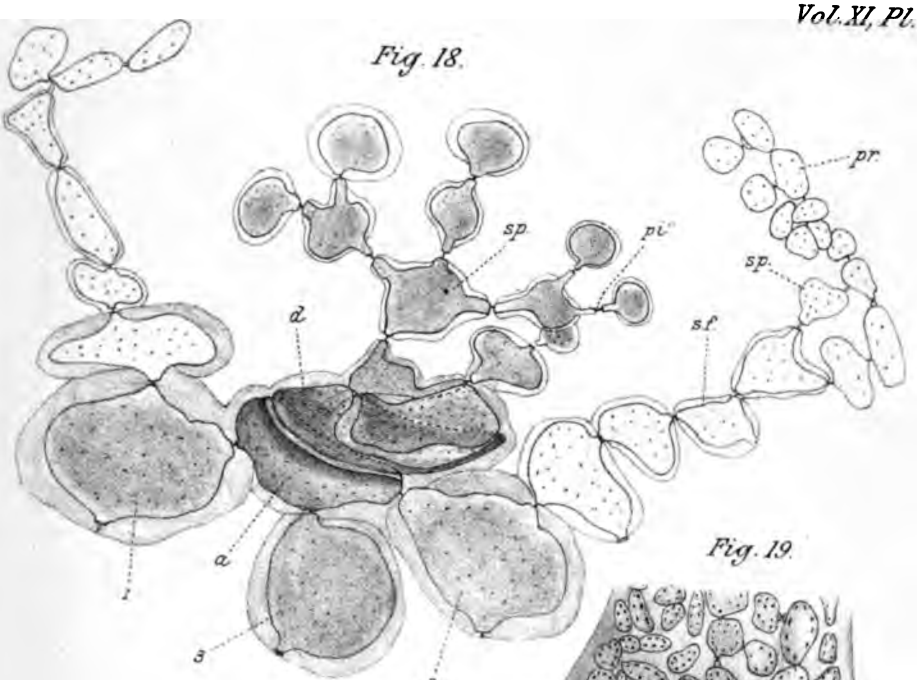

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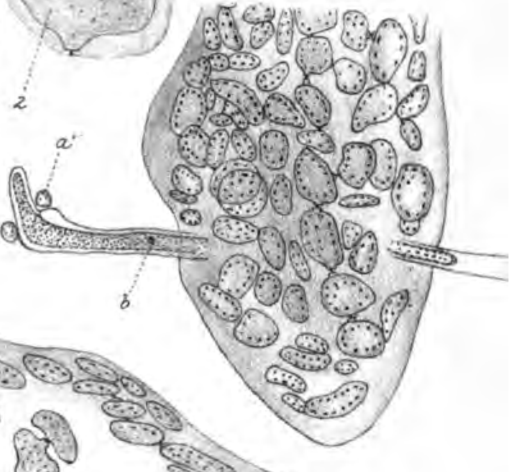

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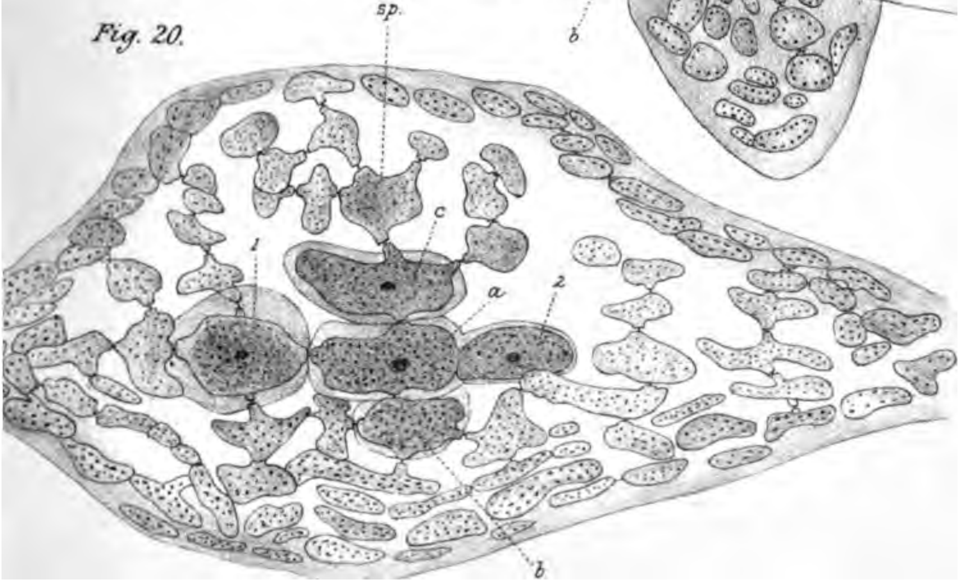

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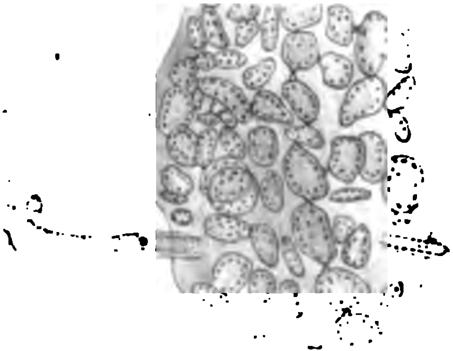
Fig. 18

Fig. 19



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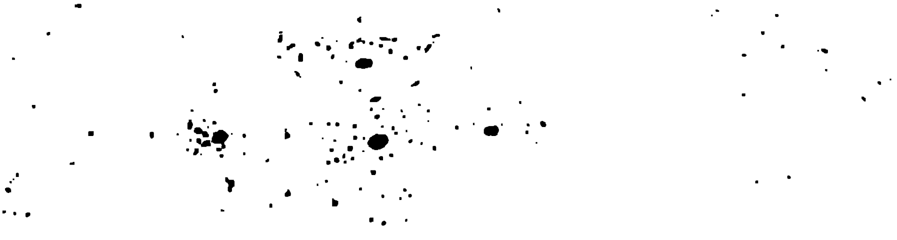

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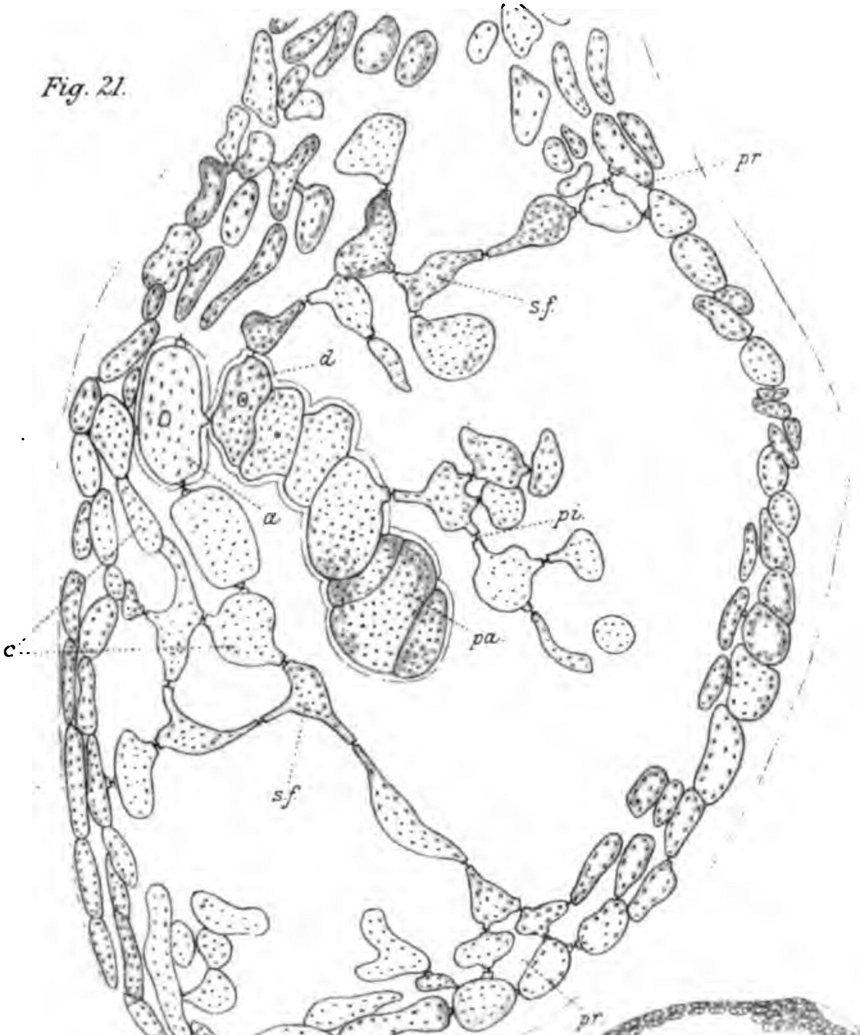

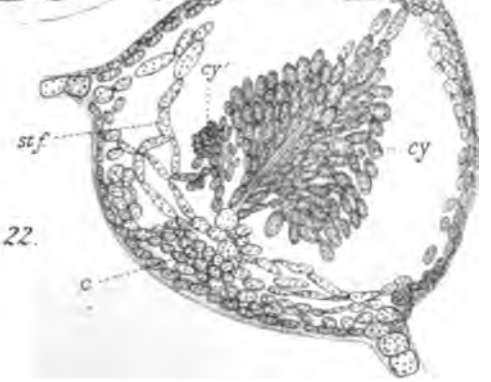

Fig. 22.

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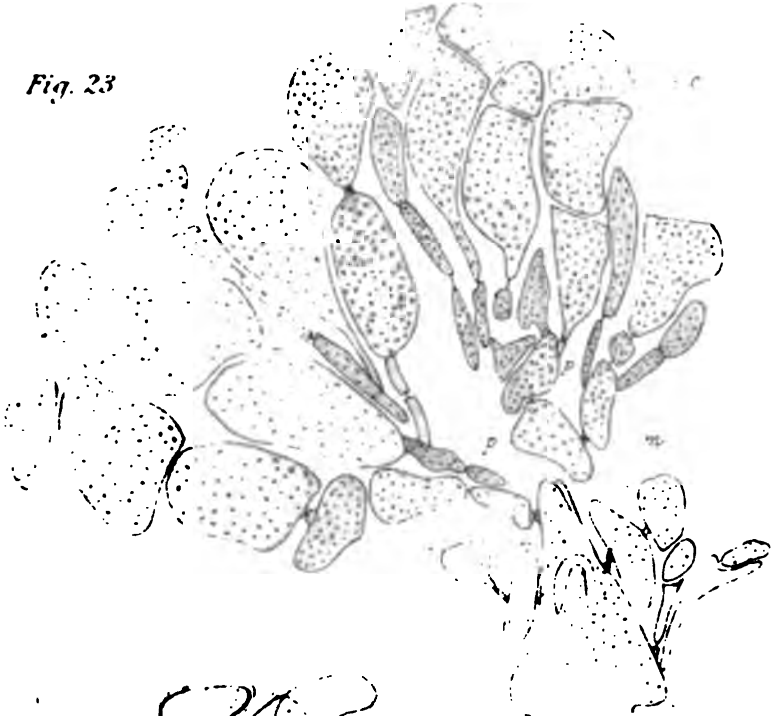
Fig. 23



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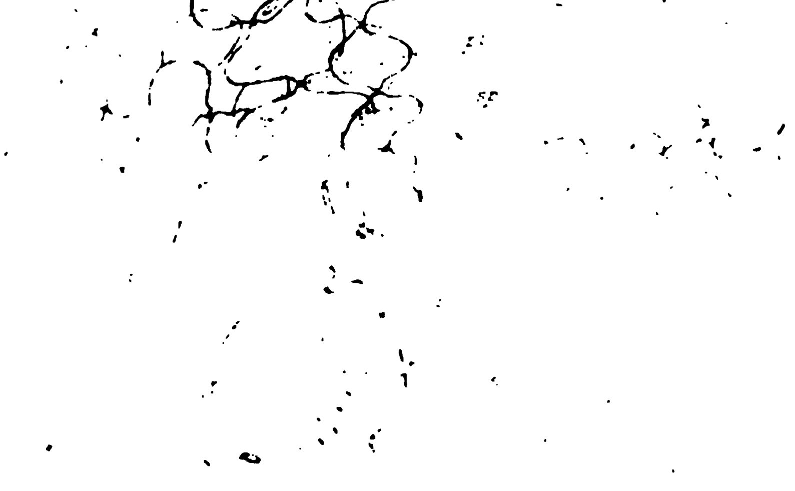

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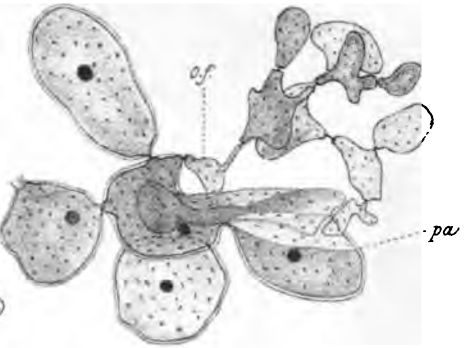

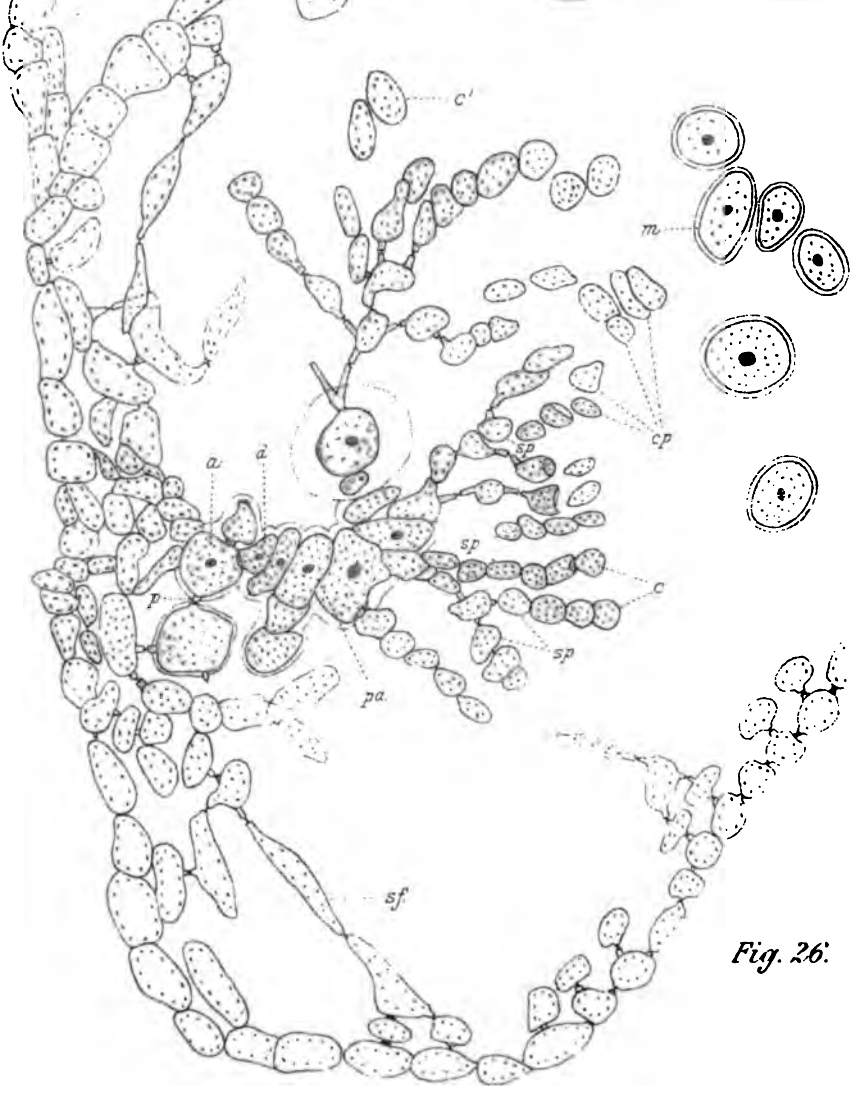

Fig. 26.

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The Evolution of Heat by Wounded Plants.

BY

HERBERT MAULE RICHARDS.

With Woodcuts 1 and 2.

THE capability of plants to respond by increased vital activities to different forms of injury is a fact which is well recognized, although the attending phenomena have not in all cases been thoroughly investigated. It is not difficult to enumerate a number of instances where, in quite dissimilar ways, a definite reaction towards abnormal conditions has been observed. There may be mentioned, for instance, the formation of callus and of corky tissue, which follows injury, or the abnormal growths of tissue attending the irritation caused by animal parasites (galls) or by parasitic Fungi. In such cases the evidences of unusual activity on the part of the plant is shown in the morphological differences of the affected tissue. From an entirely different standpoint, that of the stimulus of the ordinary functions of the cells themselves, the work of Hauptfleisch[1] affords interesting evidence. It appears from his observations that the movement of protoplasm is accelerated to an unwonted degree by injury to adjoining cells. In some plants, where under ordinary conditions no motion is observable (e.g. *Elodea*,

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[1] Pringsh. Jahrb., Vol. xxiv, p. 190, 1892.

(Annals of Botany, Vol. XI. No. XLI. March, 1897.)

Vallisneria), a marked streaming is seen after injury to neighbouring parts. But in relation to the subject of this paper, the effect of injury or other abnormal conditions on the functions of the plant as a whole is of particular interest. One of the best measures of increased activity of a plant is found in the intensity of the respiration; and this last has been determined, under a variety of irritating influences, to be temporarily considerably above the normal. For instance, under exposure to the vapour of chloroform or ether[1], or to various gases in increased or diminished pressure[2], and also after wounding[3], the CO_2-production rises markedly, and in the last-mentioned case at least subsequently falls to the normal.

It would seem then possible that all this extra work which is done under the stimulus of irritation, would necessitate a rise in the temperature of the affected parts. So it was suggested to the writer during his work on the respiration of injured plants, and so also had Pfeffer forecast the possibility of an increase in the temperature of plants through wounding[4]. To measure any rise of temperature on the wound-surface itself, it is of course quite impossible to employ a thermometer; instead of which a thermo-element, of which a description will be given later, was used. The results of the experiments attained by this method, and also on a large scale with a rough calorimeter, were sufficiently successful to warrant an account of them. The writer has again to thank Professor Pfeffer very much for his advice and criticism, and for the facilities afforded in the laboratory. In this place also the writer would express his obligation to Dr. Giessler, one of the assistants in the Botanical Institute at Leipzig, whose invaluable assistance, in the many operations which required two persons to carry out, contributed greatly to the success of the work.

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[1] Elfving, Öfversigt af Finska Vetensk-Soc. Förhandlingar, Vol. xxviii, 1886.

[2] Johannsen, Untersuch. Tübingen, I. 686.—Stich. Flora, 1891, p. 1.

[3] Richards, Respiration of Wounded Plants, Ann. of Bot., Vol. x, p. 532, 1896.

[4] Studien zur Energetik der Pflanze, 1892, footnote 2, p. 201.

It should be understood at the outset that the results herein recorded should be taken as no more than a qualitative, and not as a quantitative, determination of the heat produced by injured plants. The object of the research was, in the first place, to establish the fact of a rise in temperature, if any, following the wounding of plant-tissues; and in the second place to determine the curve of the change of temperature. While all possible pains were taken to shut out, in so far as could be, any sources of error, the extreme accuracy of Rodewald's[1] quantitative research was not so necessary.

As regards the apparatus and methods used, the thermo-electric element, in connexion with a mirror-galvanometer, was, as has already been hinted, mainly employed. The bolometric method was not used, since the former means proved sufficient for the purposes desired. A simple thermo-element similar to the form used by Dutrochet[2], was the one selected. Such a single element is by no means so delicate as is a battery of twelve or thirty-six, such as Rodewald[3] employed, but it proved sensitive enough, and was more advantageous, since the wound made by the element in the tissue under experimentation was reduced to a minimum. Such an element consists of two pieces of soft iron wire connected by a bow of German-silver wire, the points of contact being soldered by means of silver. The free ends of the iron wires are connected by means of binding screws to the wires from the galvanometer. The precise form of the points of this thermo-electric needle was only determined by experiment. At first sharp needle-points were tried, but proved ineffective for the purpose in hand, since they freed themselves too easily from the tissue in which they were imbedded. It was found that spatulate points, the idea of using which was obtained from Stahl[4], were preferable, affording as they do a firmer hold in the plant, and also a larger surface upon which the warmth may act. An element of this form, made out of iron wire of about 1 mm. and German-silver wire of about ·75 mm. in thickness, beaten out to form points about 6 mm. in width at the widest point, was the one employed (see woodcut). Before use care was taken that it should be thoroughly coated with shellac to protect it from possible chemical action, and a large part of the element, where it would be likely to come in contact with the hands or other outside object, was covered with thick strips of cork cemented on with sealing-wax.

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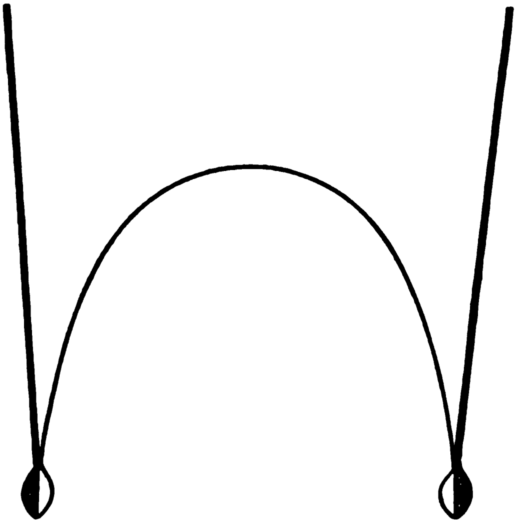
[1] Pringsheim, Jahrb., Bände xviii, p. 263, xix, p. 221, xx, p. 261.

[2] Annales des Sciences Naturelles Bot., Tome xiii, Série 2, p. 5 et seq.

[3] Pringsh. Jahrb., Band xviii, p. 276.

[4] Stahl, Ann. du Jardin Bot. de Buitenzorg, Vol. xiii, p. 143.



Woodcut 1.

Diagram of the thermo-electric element employed; somewhat reduced. The shaded portion and the outer straight wires are of iron: the inner bow of German-silver.

The galvanometer was one of the usual mirror-type, with scale and telescope, of which no especial description is necessary. The distance of the scale and telescope from the galvanometer itself was about 2·5 metres, but the telescope was sufficiently powerful to make each division of the scale appear as much as 3 millimetres across, so that a quarter or even a fifth of one division could be read with ease. There are many sources of error in the use of this instrument which require great precautions to exclude: all due care was taken to do so in so far as they might influence the work in hand. The galvanometer was naturally exceedingly sensitive to masses of iron; hence, so far as possible, all iron objects, particularly movable ones, were removed from the room. Another necessity is that the room in which the experiments are being carried on should be of constant or nearly constant temperature. This, together with a no less important condition, that of a perfectly solid foundation for the instruments, was realized in the room in which these experiments were performed; where the temperature remained for the most part exceedingly equable, within a few tenths of a degree, and the cement floor being directly on the ground, was quite free from vibration. The thermometer on the level of the table where all the plants were kept, registered a little over 24 C. For rendering the galvanometer astatic, a bar-magnet was used and so arranged that the observer at the telescope could alter its position and thus control the zero-point. The change of the declination of the magnet of the galvanometer, due to the earth's magnetism, could not be disregarded; hence before each observation a zero-point was established by cutting out the thermo-element from the circuit with an ordinary mercury commutator.

In the use of the thermo-electric element Rodewald[1] gives a number of precautions against errors, which were followed in so far as necessary in this work. The most important is the complete insulation of the element by means of an even, but not too thick, coat of hard shellac, which at the same time serves to protect the metal from the chemical action of the juices of the plant. A careful watch was kept to see that the shellac coating always remained intact, and very frequently the element was washed clean and re-lacked. The binding-screws which connect the iron wires of the element with the copper wires coming from the galvanometer were another source of trouble, since any disturbance of their constancy of temperature was followed by uncertain deflections of the galvanometer. Finally, however, they were encased in cork, which was found to thoroughly protect them from outside influences. As has already been said, a large portion of the element itself was also protected with cork and sealing-wax. The above are the most weighty sources of error as regards the galvanometer and the thermo-electric element; in the actual experiments on living plants there are other precautions to be taken, of which more will be said later.

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[1] [illegible] xviii, p. 281.

Frequent experiments established the fact that, with the scale used and at the distance at which it was placed from the mirror, one division had the value of 0·07° C. It was found for the purpose desired that water of different temperatures, measured by standard normal thermometers, was as accurate as a more complicated apparatus with oil. Alcohol was also used, and the results obtained thereby agreed with the determinations made with water. In the experiments which will be found given at the end of this paper, one division of the galvanometer scale is taken then as the equivalent of 0·07°.

In the manipulation of the plants under experimentation, there are also many precautions to be taken against possible errors. In the first place the plants must be kept in an atmosphere saturated with moisture, both to prevent their withering and also to avoid changes of temperature due to evaporation from the cut surfaces of the injured plant. It is also necessary that they should have acquired the temperature of the warm room. A day or two was always allowed before the plants brought into the warm room were used. Another point which is exceedingly important is the placing of the thermo-needles in the objects. When the needle is thrust too much into that portion of the wound which gapes open, naturally little or no effect is observed; but when it is properly arranged near the base of the cut where the sides come together, the needle-point receives the full influence of both the cut surfaces. The latter method was always practised, with the exception of a few cases where the wound gaped so widely that it was impossible; under which circumstances the needle was stuck into the tissue directly behind the cut. Why this latter was not always done will be explained in a following paragraph. It is unfortunately impossible to obtain the necessary comparison of temperature between the injured and uninjured parts without inserting the needle into the latter as well as the former, and thereby wounding it slightly. The wound is slight. however, and as oxygen of the air can scarcely gain access around the needle thus introduced, the difference is not so important as it would seem. The error, too, if any, would tend to counteract the effect of the intended wound in the injured part, and hence in a qualitative determination such as this is not serious, especially since all the objects experimented with were treated in the same manner. In order to ensure absolute certainty that the injury purposely inflicted would not influence both needle-points, two objects were used (e.g. two potatoes) which are here always referred to as the uninjured and injured objects. It was found by repeated experiments that the various plants used, if they were of the same age and had presumably been kept under the same conditions, never varied appreciably in temperature in the uninjured condition. Without going further into the description of the manipulations gone through, it is sufficient to repeat that the various objects investigated were treated in as nearly as might be the same manner, and any errors arising thereby largely equalized; which it will be appreciated is the most important desideratum in a purely *qualitative* determination The writer recognizes, of course, that in a *quantitative* estimation of the amount of heat produced a much finer degree of accuracy would be required, such as Rodewald[1] employed in his admirable researches

As a control for the thermo electric method, a series of experiments were carried on with a simple form of a calorimeter, by which the temperature-curve of a mass of plants could be determined The results were of a confirmatory nature. The apparatus consisted simply of two bell-glasses of about 1½ litres capacity, protected all around with a thick layer of cotton-wadding. The bell-glasses were also supplied with thermometers, which had previously been compared, and a small aperture, loosely stopped with a bit of cotton to prevent any rapid circulation, was provided for the admission of air. Inside the bell-glasses were also dishes of KOH for the absorption of the CO_2 respired, and above these the plants were supported. After filling both of the bell-glasses, a day was allowed for them to become equalized in temperature. If readings taken in the evening and again the next morning both showed no difference between the two bell-glasses, the plants in one of them were cut and then returned as quickly as possible. After this the difference between the temperatures in the two bell-glasses registered the effect of the injury, and readings taken from hour to hour showed the curve. It is unnecessary to say that these experiments were carried on in the room the temperature of which only on one or two occasions varied within a few tenths of a degree. The difference in the temperatures between some of the experiments is due to a difference in location of the bell-glasses. When on a higher level they were naturally somewhat warmer. For this reason the thermometers were carefully adjusted to the same level and never moved during the course of any one experiment. The amount of heat arising from the absorption of the CO_2 by the KOH was not regarded, although of course it was greater in the injured than in the uninjured plants. But this is counterbalanced by the fact that the warmer bell-glass must also lose more by radiation than the cooler. The apparatus was not intended to be more than approximately accurate, and the application of such corrections would not be worth while for the results required.

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[1] Pringsh. Jahrb., l. c.

Although potatoes were used more than any other plant, a variety of others representing different sorts of tissue were experimented with. But potatoes were more convenient, and also had the great advantage of resisting the attacks of fungi better than anything else. The writer had also experimented with them in regard to their respiration in the injured condition, and it was desirable to be able to compare the results of this with those of the previous investigation. The experiments with potatoes will, then, be first taken up.

It was never found that there was any difference in temperature between the cut surfaces and the uninjured potato immediately after injury; it was not until the second observation was made, usually some two hours after wounding, that any indication of increasing warmth in the injured plant was noticeable. The amount of increase was not found to be great at this time, being only about 0·07° C., but there is a steady increase after this. That there should be no sudden increase immediately after injury is to be expected. The cause of the large CO_2-production at this time has been shown[1] to be a physical one. The maximum temperature is not reached until some twenty-four hours after injury, a period corresponding to that of the respiration-intensity. As might be expected, there is some range of variation in the reaction of individual potatoes. Many things might lead to differences: as, for instance, a difference in the metabolic activity due to different age. It was usually found, however, that potatoes procured at the same time, i.e. which had presumably been gathered simultaneously and kept under the same conditions, gave pretty uniform results. The difference between old potatoes of the previous year and new ones was marked, as will be discussed a little later. One source of error which was to be guarded against was the use of unhealthy potatoes. It was found that sometimes potatoes with only a small rotten spot, which had no influence on the general temperature of the tuber, would further rot in the warm, moist atmosphere in which they were kept, until the decayed portion would affect the temperature of almost the whole potato. This is particularly insidious in that the increase of temperature from the rotting of the potato would simulate the rise following injury. Several series were discarded for this reason, and great care was taken to have none but healthy potatoes.

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[1] Richards, Respiration of Wounded Plants, Ann. Botany, Vol. x, p. 544, 1896.

After an experiment was completed, the tubers were cut in pieces and examined for any decayed spots. The surfaces of the wounds made in the potatoes never showed any signs of decay, presenting only the layer of corky tissue, quite dry and hard, over the surface.

The highest maximum ever noted was a temperature-difference of the injured and uninjured potatoes of almost 0·4° C. The return to the normal temperature after the maximum is slower than the ascending curve. There is a distinct drop in the temperature a few hours after the maximum, followed by a much slower decrease; until, by the fourth or fifth day after the injury was inflicted, no difference was to be determined with the apparatus used. The actual rise in temperature is not, of course, so very much; but when the normal temperature of potatoes in relation to the surrounding medium is considered, it is not to be despised. Repeated experiments showed that at the temperature of the warm room, namely about 24° C., old potatoes of the previous year showed a difference between the living and dead tissue of 0·16° C. In new potatoes only recently out of the ground, the plus temperature under the same conditions of temperature and moisture was somewhat lower, viz. 0·13° C. That the old potatoes have a higher temperature than the new ones is not remarkable, since the former are just emerging from their resting period, while the latter are just entering a period of quiescence where the metabolic activity is naturally low. A similar difference was found in the reaction to injury where the young potatoes did not respond so actively to the stimulation of the wound (compare Expts. 7, 9, and 10).

The relation which the rise of temperature bears to the increased respiratory activity after injury is naturally not to be directly estimated; since in this case the local warmth only is measured, while in the experiments on respiration it was the total effect which was determined. Approximately speaking, the temperature directly on the surface of a wound, or just below it, at the time of its maximum is about twice as great as the normal plus temperature of a living over a dead potato. Thus the temperature of a living uninjured potato being $0{\cdot}17^\circ$ C. over that of the surrounding objects, the temperature on the cut surface of a wounded potato will at its maximum be from $0{\cdot}20^\circ$ C to $0{\cdot}25^\circ$ C higher than that of the uninjured plant. But it must be remembered that in these conclusions no allowance has been made either for the conduction of heat by the tissue of the potato itself or for radiation into the air, which must, of course, amount to something and must increase as the temperature of the tissue increases. In relation to this point and also the other regarding the comparison of the temperature with the respiration, it is exceedingly important to have some idea of how far the surrounding tissue of the potato is sympathetically affected in the increased warmth following injury.

It will be seen from Experiments 11 and 12 that the influence of the wound does not extend very far back from the point of irritation. There is in most cases a very rapid falling off at a distance of only a few millimeters from the wound; and at a distance of two centimeters there is no perceptible difference. It is evident, then, that the real heat-producing processes which follow injury must be comparatively local, for the conduction of heat by the tissue of the potato naturally increases the distance at which a temperature difference is distinguishable. In order to obtain a clearer understanding of this question, experiments were made to determine an approximate idea of the conductivity of potato-tissue for heat. Two potatoes in separate vessels were half buried in sand which was in one at the temperature of the air, namely 24·2 C.; while in the other it was kept during the time of the experiment nearly ten degrees higher. The needles were thrust into the two potatoes at a distance of 3[illegible] mm. from the surface of the sand, and in fifteen minutes the galvanometer indicated an increase in temperature of the warmed potato amounting to 0·25 C. By another experiment it was also found that two potatoes which showed by the galvanometer a difference in temperature of almost exactly 2 C., took five and a half hours to become equalized, being kept the while at the temperature of the cooler of the two potatoes, namely 24·1° C. It is evident from these experiments that while the conductivity of the tissue for heat may not be very rapid in comparison with other bodies, it is not to be disregarded. Hence it is not too much to say that the actual heat-reaction of the wound itself appears less localized than it in reality is. It is, of course, impossible to separate these two factors, the actual warmth and the conducted warmth; but having found that, even including the latter, the tissue does not seem to show any increase in temperature beyond 20 m. from the wound, it is safe to say that the actual wound-reaction is probably considerably inside of even this.

In the experiments with the two bell-glasses packed with cotton-wadding, the rise in temperature caused by a large mass of potatoes—500–600 grams—may be plotted on a curve which has much the same course as that determined by the thermo-electric method (see Woodcut 2, p. 63). Naturally it is not possible to compare the actual temperatures indicated by the two methods, for neither is quantitative. It is to be expected that the rise indicated by a large mass of potatoes should be higher than that obtained from a single tuber, and such is the case. That, as measured by this method, it should be proportionally greater is not to be looked for; as the indirect way of measuring the heat and the loss by radiation gives no accurate knowledge of the actual amount produced. The method is of value, however, in that in the two bell-glasses are two equal masses of potatoes under exactly similar conditions, except that in one they are injured while in the other they are normal. The rise to the maximum takes some twenty-four hours after the potatoes have been cut and returned to the bell-glass, and is about 0·9° C. higher than the temperature given by the uninjured potatoes, the latter showing ordinarily a plus temperature of 0·4° C. above that of the surrounding air. The return to zero of difference follows in the course of a few days. Such a method as this, if strictly followed out calorimetrically, would give results which could be better compared with the experiments made on respiration, since both methods deal with the total reaction on masses of tissue where the effect cannot be localized.

None of the other plants employed were investigated so thoroughly as potatoes, but enough to show that they too gave a distinct temperature-reaction after injury. Radishes were experimented with once or twice, and showed, in so far as the experiments could be carried, a reaction similar to that of potatoes. One experiment with the bell-glasses herein recorded (Expt. 23) remained trustworthy until after the maximum had been reached, but later it had to be abandoned as the radishes began to decay. The same trouble occurred with the other experiments, and for this reason radishes were no longer used. It was difficult at the time of year—June and July when these experiments were made, to obtain carrots of sufficient size for use; and they also have the disadvantage that the wounds when made gape open very widely after a few hours. Nevertheless a series was successfully finished, and showed very much the same results as those obtained with potatoes, although the temperature was lower (Expt. 15). This last fact may perhaps be explained by the circumstance of the gaping of the wound, which thereby allowed more loss of heat by radiation. The maximum comes at a somewhat later period, and the return to normal temperature somewhat slower than with potatoes, but this corresponds with the respiration-curve previously determined[1]. In the bell-glasses the highest maximum yet mentioned was found, being 1·35° C. (Expt. 29), and as well the highest plus temperature of the normal tissue was indicated by both methods. By the thermo-electric method which gives the actual temperature of a single carrot, it was found under normal conditions to be 0·2° C. (Expt. 16). It will be remembered that the normal respiration of carrots is very high, being about 15 mg. per hour. The swollen stem of the Kohl Rabi (*Brassica oleracea* var. *gongylodes*), and the fruit of the Cucumber, also gave temperature-curves with the thermo-electric apparatus (Expts. 13, 14, 20), but were not tried with the bell-glasses.

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[1] Richards, Respiration of Wounded Plants, l. c., Expts. 14, 15.

Leaves afforded an entirely different type of tissue. Ordinarily speaking these are not well adapted for the use of a thermo-element. The thick leaves of *Aloe* were experimented with in this way, although without success; but with onion-bulbs very interesting results, of which more will be said later, were obtained. With the bell-glasses ordinary leaves could be employed: experiments with those of *Liriodendron* and *Diervilla* gave very satisfactory results (Expts. 24, 25). The maximum, as would be expected, was reached much more rapidly than with massive tissues. In four and a half hours the injured leaves, in the case of *Liriodendron*, were 0·75° C. warmer than the uninjured, the latter showing an average temperature of about 0·4° C. over that of the air. By the next day there was little difference, and that speedily diminished as the injured leaves died.

Of all the plants investigated onion-bulbs gave a far stronger reaction than any other. With the bell-glasses the average maximum of two experiments (Expts. 26 and 28) was 3·3° C. This high temperature continued for some time; and it was not until the fifth day that it sank again to the normal plus temperature of about 0·5° C. In one experiment (Expt. 27) it will be noticed that the maximum was much lower; but, at the same time, the temperature of these onions in the uninjured state was very much higher than in the other two experiments, being 1·2° C. above air-temperature. This accounts for the low maximum in the injured condition, and is probably to be explained by a difference in metabolic activity of the onions. It may be mentioned that the onions used in this experiment did not come from the same source as the others. With the thermo-element, however (Expts. 17 and 19), no remarkably high temperature was detected in the cut, or rather immediately behind it; since in onions the wounds gape so that it is impossible to stick the needle advantageously into the cut itself. This fact was puzzling until, by subsequent experiment, it was found that the whole onion is more or less, though somewhat irregularly, affected, and that at so great a distance as 45 mm. from the wound a temperature of 0·17° C. over that of the uninjured bulb was found, when it was only 0·28 C. in the wound itself. It is, then, easy to believe that the small onions used in the bell-glass experiments, and injured so severely as by quartering, should give a comparatively violent reaction.

From the above discussion of the results of the experiments it is evident that there is a distinct temperature-reaction, more or less variable, which manifests itself in plants after injury. This reaction is in fact a *fever*, if one may use the term, brought about by the increased vital activities of the wounded plants. In considering the significance of this 'wound-fever' one should also bear in mind the other phenomena which attend injury. Attention has already been called to these in the introduction to this paper. Of the greatest interest is the consideration of the relation which the increase in respiration bears to the rise in temperature. The respiration-curve was found to be at its highest, in potatoes for instance, about twenty-four hours after wounding; and it is at the same time that the maximum of the temperature-curve is indicated (Woodcut 2). In the same way, as has already been said the phenomena go hand in-hand in the case of leaves. The return of the temperature to the normal is, it is true, somewhat more rapid than that of the respiration, at least as far as was indicated by the method used; but after the fourth or fifth day it will be remembered that even the respiration is only very slightly above the normal[1]. Theoretically speaking, the rise in respiration, representing as it does an increase of chemical work in the tissues of the plant, demands a rise also of temperature, and it is interesting to find that, as a matter of fact, such is the case. Even granting that the respiratory processes are alone responsible for all the heat normally produced in a plant, the temperature could hardly be expected to increase correspondingly with the respiration. The writer would again call attention to the fact that by radiation and so

[1] See Richards, Respiration of Wounded Plants, l. c.

forth an appreciable amount of the produced heat must be lost. It is, however, evident from the experiments in which the action of the air was prevented by the application of clay, that the phenomena, as regards both respiration[2] and temperature (Expt. 12*a*), are dependent on a supply of oxygen, since in these cases a marked diminution of the reaction was noticeable.

Of interest in this connexion are the results of Eriksson[1], who has shown that the amount of heat produced during intra-molecular respiration is but a fraction—a tenth or thereabouts—of that which is formed when the same plants are respiring under normal conditions. It is not to be wondered at then that after injury, when the respiration and hence also the vital activities of plants are greatly stimulated, a rise of temperature should also be found.

But it is not a simple question of how much heat should be produced by the formation of a given amount of CO_2, since it is living and not dead material which concerns us. There are, no doubt, many other changes which take place; the very fact which has already been shown[2] that apparently more O_2 is absorbed than is required for the amount of CO_2 produced shows that oxidizing reactions may take place which, although they do not influence the amount of CO_2 respired, might possibly give rise to temperature-changes. Yet in addition to these there are no doubt other phenomena attending injury which are not to be measured directly by their chemical effect. For instance, the acceleration of protoplasmic currents which Hauptfleisch[3] has described, affords direct evidence of a disturbance of the normal condition of the cell-contents. It would be interesting to know from the point of view of time how these phenomena are related to those which may be determined in a physical or chemical manner: whether the accelerated protoplasmic movements precede—which perhaps is not impossible—or follow the other phenomena. From the observations of Hauptfleisch it would seem that, while the more rapid circulation is not general through the whole of an injured plant. it is propagated with a speed and to a distance which is not commensurate with the apparently local character of the heat-reaction, so that it would appear that the former perhaps precedes the latter.

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[1] Über Wärmebildung und intramoleculare Athmung der Pflanzen, Untersuch. Bot. Inst. zu Tübingen, Band I, p. 105 et seq.

[2] See previous paper, l. c. Table on p. 548.

[3] Prings. Jahrb., l. c.

That plants should, like animals, respond to the stimulus of an injury by an attempt to rally from it is not surprising; but that this rallying should be accompanied by somewhat the same symptoms is a matter of no little interest. It is true that the reaction is, absolutely, not so marked with a plant as with the higher animal; but when one considers that in the former case the cells and tissues are not so interdependent as in the latter, and that the whole scale of vitality is keyed to a lower pitch, this cannot be wondered at.

It should be remembered, however, that compared with the ordinary temperature of plants over the surrounding medium, the rise of temperature after injury is as great, if not greater, than in animals. Large or small the reaction is, however, sufficiently well marked to justify institution of a comparison between the rise in temperature following the injury of plants and the fever following the wounding of animals, both being due to the exerting of the vital forces of the organism to recover from the shock.

The main results of these experiments, as far as is to be judged from the objects experimented with, may be stated, in brief as follows:

1. That a certain rise in temperature of the adjacent tissue follows on the wounding of plants.

2. That this 'fever reaction' runs a definite course, attaining its maximum some twenty-four hours after injury, so that a curve plotted from it corresponds in the main to that of the respiration intensity under similar conditions (Woodcut 2).

3. That the maximum in all the plants investigated was between two and three times the ordinary plus temperature of the plant.

4. That in potatoes (i.e. massive tissues) the effect is local, but that in onion bulbs (i.e. leaves) a much greater extent of tissue is sympathetically affected.

A.

The experiments in this section were all made with a thermo-electric element attached to a galvanometer of the form and after the manner already described. In all cases the material used had been kept at least twenty-four hours in the warm room before being used. Hence no mention of this is made under the individual experiments. The readings of the galvanometer are given in divisions and fractions of divisions of the scale employed, and are corrected to the actual zero-point of galvanometer at the time the observations were made. 1 division of galvanometer scale = 0·07° C.

EXPERIMENT 1.

Two potatoes 7–8 cm. long. In uninjured condition show equal temperature. One cut at 4.00 p.m., the other left uninjured.

Duration of Experiment.	Temperature in room.	Deflec. of Galvan.	Equiv. in °C.
First day 4.00 p.m.	24°·50	0·0	0°·00
„ 6.00 „	24°·40	1·1	0°·08
„ 8.45 a.m.	24°·10	2·7	0°·19
„ 12.45 p.m.	24°·30	4·0	0°·28
Second day 4.30 ,,	24°·30	2·8	0°·20
„ 9.30 a.m.	24°·20	1·2	0°·08
Third day 9.30 „	24°·40	0·5	0°·03
Fourth day 9.30 „	24°·40	0·0	0°·00

EXPERIMENT 2.

Two potatoes 7–8 cm. long. No difference in temperature in uninjured state. One cut at 4.00 p.m.

Duration of Experiment.	Temperature in room.	Deflec. of Galvan.	Equiv. in °C.
First day 4.00 p.m.	24°·50	0·0	0°·00
„ 6.00 „	24°·40	1·5	0°·09
„ 8.30 a.m.	24°·10	2·7	0°·19
„ 12.30 p.m.	24°·30	4·5	0°·31
Second day 4.30 ,,	24°·30	3·0	0°·21
,, 9.30 a.m.	24°·20	0·5	0°·03
Third day 9.30 „	24°·40	0·3	0°·02
Fourth day 9.30 „	24°·40	0·0	0°·00

EXPERIMENT 3.

Two potatoes 8-9 cm. long. In uninjured condition showed no difference of temperature. One cut at 8.45 a.m.

Duration of Experiment.		Temperature in room.	Deflec. of Galvan.	Equiv. in °C.
First day	8.45 a.m.	24°·10	0·0	0°·00
"	12.00 noon	24°·20	1·0	0°·07
"	5.00 p.m.	24°·25	2·0	0°·14
Second day	9.00 a.m.	24°·10	3·0	0°·21
"	4.45 p.m.	24°·10	2·2	0°·15
Third day	9.30 a.m.	24°·30	0·8	0°·06
Fourth day	9.30 "	24°·30	0·0	0°·00

EXPERIMENT 4.

Two potatoes 6-7 cm. long. In uninjured condition showed no difference of temperature. One cut at 9.00 a.m.

Duration of Experiment.		Temperature in room.	Deflec. of Galvan.	Equiv. in °C.
First day	9.00 a.m.	24°·10	0·0	0°·00
"	12.00 noon	24°·20	1·0	0°·07
"	4.45 p.m.	24°·30	2·0	0°·14
Second day	9.30 a.m.	24°·00	3·5	0°·23
"	4.00 p.m.	24°·20	2·8	0°·20
Third day	9.00 a.m.	24°·20	1·0	0°·07
"	4.00 p.m.	24°·10	0·6	0°·04
Fourth day	9.00 a.m.	24°·30	0·2	0°·01
Fifth day	9.00 "	24°·10	0·0	0°·00

EXPERIMENT 5.

Two potatoes 8 cm. long. Uninjured equal in temperature. One cut at 9.30 a.m.

Duration of Experiment.		Temperature in room.	Deflec. of Galvan.	Equiv. in °C.
First day	9.30 a.m.	24°·20	0·0	0°·00
„	12.15 p.m.	24°·20	0·8	0°·06
„	3.00 „	24°·25	1·6	0° 11
„	4·50 „	24°·30	3·0	0°·21
Second day	9.30 a.m.	24°·00	3·8	0°·27
„	4.00 p.m.	24°·00	2·5	0°·17
Third day	9.00 a.m.	24°·20	0·6	0°·04
„	4·15 p.m.	24°·30	0·5	0°·03
Fourth day	9.00 a.m.	24°·10	0·3	0°·02

EXPERIMENT 6.

Two potatoes 7 cm. long. Uninjured equal in temperature. One cut at 9.00 a.m.

Duration of Experiment.		Temperature in room.	Deflec. of Galvan.	Equiv. in °C.
First day	9.30 a.m.	24°·10	0·0	0°·00
„	12.00 noon	24°·10	0·7	0°·05
„	4·30 p.m.	24°·30	1·5	0°.10
Second day	9.30 a.m.	24°·20	4·3	0°·30
„	4.00 p.m.	24°·30	3·0	0°·21
„	6.30 „	24°·35	2·2	0°·15
Third day	9.00 a.m.	24°·20	1·0	0°·07
„	4.00 p.m.	24°·30	0·6	0°·04
Fourth day	9.00 a.m.	24°·10	0·0	0°·00

EXPERIMENT 7.

Two potatoes 10 cm. long. These potatoes were of another lot than the ones previously tried. In uninjured condition no difference in temperature. One cut at 9.00 a.m.

Duration of Experiment.		Temperature in room.	Deflec. of Galvan.	Equiv. in °C.
First day	9.00 a.m.	24°.40	0·0	
„	11.30 „	24°.50	1·5	0°.10
„	3.45 p.m.	24°.30	2·5	0°.17
„	6.00 „	24°.30	3·3	0°.23
Second day	9.00 a.m.	24°.10	4·5	0°.31
„	11.00 „	24°.20	5·5	0°.38
„	4.00 p.m.	24°.30	3·7	0°.26
Third day	9.00 a.m.	24°.30	2·7	0°.18
„	12.00 noon	24°.40	2·0	0°.14
„	4.00 p.m.	24°.40	1·5	0°.10
Fourth day	12.00 noon	24°.10	0·8	0°.06
Fifth day	12.00 „	24°.20	0·3	0°.02

EXPERIMENT 8.

Two potatoes 9 cm. long; of same lot as Experiment 7. No difference in temperature in uninjured condition. One cut at 9.00 a.m.

Duration of Experiment.		Temperature in room.	Deflec. of Galvan.	Equiv. in °C.
First day	9.00 a.m.	24°.40	0·0	0°.00
„	11.30 „	24°.50	1·5	0°.10
„	4.00 p.m.	24°.30	2.3	0°.16
„	6.15 „	24°.30	3·3	0°.23
Second day	9.00 a.m.	24°.10	4·0	0°.28
„	11.00 „	24°.20	5·3	0°.37
„	4.00 p.m.	24°.30	4·2	0°.29
Third day	9·00 a.m.	24°.30	3·0	0°.21
„	12.00 noon	24°.40	2·3	0°.16
Fourth day	12·00 „	24°.40	1·3	0°.10
Fifth day	12·00 „	24°.20	0·4	0°.03

EXPERIMENT 9.

Two potatoes 5–6 cm. long. These were young potatoes of the new year's crop, unlike the one previously experimented with. In the uninjured condition they showed no difference in temperature. One cut at 9.00 a.m.

Duration of Experiment.		Temperature in room.	Deflec. of Galvan.	Equiv. in °C.
First day	9.00 a.m.	24°·30	0·0	0°.00
„	11.00 „	24°·30	0·6	0°·04
„	3.00 p.m.	24°·10	1·6	0°·11
Second day	9·30 a.m.	24°·20	2·0	0°·14
„	1.00 p.m.	24°·50	1.8	0°.13
Third day	12.00 noon	24°·30	1·8	0°·13
„	3·30 p.m.	24°·30	1·5	0°·10
Fourth day	4.00 „	24°·10	0·0	0°.00

EXPERIMENT 10.

Two potatoes 5–6 cm. long. Same lot as used in Experiment 9. No difference in temperature in uninjured condition. One cut at 9.30 a.m.

Duration of Experiment.		Temperature in room.	Deflec. of Galvan.	Equiv. in °C.
First day	9.30 a.m.	24°·30	0·0	0°.00
„	11.15 „	24°·30	0·4	0°·03
„	3·15 p.m.	24°·10	0·8	0°·06
„	6.00 „	24°·20	1·2	0°·08
Second day	9.30 a.m.	24°·20	2·3	0°·16
„	3.00 p.m.	24°·50	1·6	0°·11
Third day	9.00 a.m.	24°·30	1·2	0°·08
„	3·30 p.m.	24°·20	0·8	0°.06
Fourth day	4.00 „	24°·10	0·3	0°·02

EXPERIMENT 11.

To determine the distance at which the increase of temperature may be noticed. Two potatoes, one of which was cut in the usual way, were kept until the injured one had attained its maximum temperature.

	Deflec. of Galvan.	Equiv. in Celsius °	Temperature in room.
In wound	3·0	0·21	24°·30
5 mm. distant	1·8	0·11	
11 mm. „	1·0	0·07	
15 mm. „	0·7	0·05	
20 mm. „	0·0	0·00	

EXPERIMENT 12.

For the same purpose as Experiment 11. Potatoes treated in the same way.

	Deflec. of Galvan.	Equiv. in Celsius °	Temperature in room.
In wound	3·0	0·21	24°·30
0·5 mm. distant	2·2	0·15	
3·5 mm. „	2·0	0·14	
8·0 mm. „	1·8	0·13	
1·0 mm „	1·6	0·11	
1·3 mm. „	1·0	0·07	
1·7 mm. „	0·5	0·03	

EXPERIMENT 12 *a*.

To determine the effect of exclusion of air from the wounded parts. Two potatoes, one of which was cut and the cuts immediately pressed together and covered with clay. No deflection of the galvanometer was observed immediately subsequent to injury. After standing twenty-four hours clay removed and determination made.

		Temperature in room.	Deflec. of Galvan.	Equiv. in C.
July 20	9·30 a.m.	24·30	1·0	0·07
„	12·30 p.m.	24·5	1·7	0·12
„	5·00 p.m.	24·30	2·4	0·17

EXPERIMENT 13.

Two specimens of kohl-rabi (*Brassica oleracea* var. *gongylodes*) 7–8 cm. diameter. Leaves had been removed, but wounds entirely healed. In uninjured condition showed no difference in temperature. One cut at 9.00 a.m.

Duration of Experiment.		Temperature in room.	Deflec. of Galvan.	Equiv. in °C.
First day	9.00 a.m.	24°·20	0·0	0°.00
„	11.30 „	24°·20	0·8	0°.06
„	4.00 p.m.	24°·20	2·0	0°.14
Second day	9.00 a.m.	24°·30	3·3	0°.23
„	12.00 noon	24°·20	2·6	0°.18
„	3.00 p.m.	24°·40	2·0	0°.14
Third day	11.30 a.m.	24°·30	1·0	0°.07
Fourth day	9.00 „	24°·10	0·4	0°.03
Fifth day	9.30 „	24°·20	0·0	0°.00

EXPERIMENT 14.

Two specimens of kohl-rabi (*Brassica oleracea* var. *gongylodes*) 8 cm. in diameter. As above. Uninjured, no difference in temperature. One cut at 9.00 a.m.

Duration of Experiment.		Temperature in room.	Deflec. of Galvan.	Equiv. in °C.
First day	9.00 a.m.	24°·20	0·0	0°.00
„	11.45 „	24°·20	1·3	0°.09
„	4.15 p.m.	24°·20	2·8	0°.20
Second day	9.00 a.m.	24°·30	3·5	0°.24
„	3.00 p.m.	24°·40	3·0	0°.21
Third day	9.00 a.m.	24°·35	1·8	0°.12
„	11.30 „	24°·30	1·3	0°.09
Fourth day	9.00 „	24°·10	0·2	0°.01
Fifth day	9.30 „	24°·20	0·0	0°.00

EXPERIMENT 15.

Two carrots (*Daucus Carota*) about 15 cm. long. Had been thirty-six hours in warm room, and showed no difference of temperature before injury. One cut at 9.00 a.m.

Duration of Experiment.		Temperature in room.	Deflec. of Galvan.	Equiv. in °C.
First day	9.00 a.m.	24°·20	0·0	0°.00
"	11.00 "	24°·25	1·3	0°.09
"	3.00 p.m.	24°·20	2·5	0°.17
"	5.30 "	24°·10	2·8	0°.20
Second day	9.00 a.m.	24°·40	3·3	0°.23
"	12.00 noon	24°·30	3·6	0°.25
"	3.30 p.m.	24°·30	3·3	0°.23
"	6.00 "	24°·30	3·3	0°.23
Third day	9.00 a.m.	24°·10	1·8	0°.13
"	3.00 p.m.	24°·20	1·6	0°.11
Fourth day	9.00 a.m.	24°·20	0·6	0°.04
Fifth day	9·00 "	24°·30	0·2	0°.01

EXPERIMENT 16.

Comparison of living and dead tissues of various plants. The dead plants were killed with boiling water. The temperature given is the natural + temperature of the plants.

		Deflec. of Galvan.	Equiv. in °C
Potatoes (old)	a	2·3	0·16
"	b	2·5	0·17
Potatoes new	c	2·0	0·14
"	d	2·0	0·14
Onion		1·7	0·12
Carrot		2·8	0·20

EXPERIMENT 17.

Two onions (*Allium Cepa*) diameter about 6 cm. In warm room three days before using. One cut at 10.00 a.m. The wounds gape so that the needle was always inserted a little behind the cut surface.

Duration of Experiment.		Temperature in room.	Deflec. of Galvan.	Equiv. in °C.
First day	10.00 a.m.	24°·20	0·0	0°·00
„	12.00 noon	24°·20	1·6	0°·11
„	3.00 p.m.	24°·30	3·2	0°·22
„	6.00 „	24°·15	4·0	0°·28
Second day	9.00 a.m.	24°·10	4·0	0°·28
„	12.00 noon	24°·00	4·3	0°·30
„	5.00 p.m.	24°·15	4·0	0°·28
„	6.00 „	24°·20	3·0	0°·21
Third day	9.00 a.m.	24°·40	2·3	0°·16
„	12.00 noon	24°·50	2·0	0°·14
„	2.00 p.m.	24°·40	1·6	0°·11
Fourth day	9.00 a.m.	24°·50	1·0	0°·07
„	5.00 p.m.	24°·30	1·0	0°·07
Fifth day	9.00 a.m.	24°·30	0·6	0°·04
„	5.00 p.m.	24°·40	0·3	0°·02

EXPERIMENT 18.

Showing that the whole tissue of the onion is sympathetically affected. Two onions, one of which was wounded by cutting. Allowed to remain twenty-four hours until maximum reached. Temperature, 24°·40.

Distance from wound.	Deflec. of Galvan.	Equiv. in °C.
1 mm.	4·0	0·28
2·4 mm.	3·0	0·21
3·5 mm.	3·0	0·21
10·0 mm.	3·3	0·23
16·0 mm.	3·0	0·21
45·0 mm.	2·5	0·17

EXPERIMENT 19.

Two onions (*Allium Cepa*) about 5 cm. in diameter. As in Experiment 17. One cut at 9.30 a.m.

Duration of Experiment.		Temperature in room.	Deflec. of Galvan.	Equiv. in °C.
First day	9.30 a.m.	24°·20	0·0	0°.00
„	12.00 noon	24°·20	1·5	0°.10
„	3.00 p.m.	24°·30	3·5	0°.24
„	6.00 „	24°·15	4·0	0°.28
Second day	9.00 a.m.	24°·10	4·0	0°.28
„	12.00 noon	24°·10	4·0	0°.28
„	3.00 p.m.	24°·00	3·8	0°.27
„	6.00 „	24° 00	3·3	0°.23
Third day	9.00 a.m.	24°·40	2·6	0°.18
„	12.00 noon	24°·50	2·5	0°.17
„	5.00 p.m.	24°·40	1·8	0°.12
Fourth day	9.00 a.m.	24°·50	1·0	0°.07

EXPERIMENT 20.

Two cucumbers (*Cucumis sativus*) about 12 cm. long. Had been in warm room two days. Showed no difference in temperature before injury. One cut at 9.00 a.m.

Duration of Experiment.		Temperature in room.	Deflec. of Galvan.	Equiv. in °C.
First day	9.00 a.m.	24°·20	0·0	0 00
„	12.00 noon	24°·20	1·0	0 07
„	3.00 p.m.	24°·30	3·0	0 ·21
„	6.00 „	24°·40	2·3	0 16
Second day	9.00 a.m.	24°·50	2·5	0 17
„	6.00 p.m.	24°·50	1·3	0 09
Third day	9.00 a.m.	24°·40	0·3	0 02
„	5.00 p.m.	24°·50	0·3	0 02
Fourth day	9.00 a.m.	24°·50	0·0	0 00

j

B.

The experiments recorded in this section are comparisons of the temperature of two bell-glasses, properly packed with cotton wool, one containing the uninjured, the other the injured plants. The bell-glass containing the normal plants is always designated as bell-glass A, the other as bell-glass B. When it was desired to determine the ordinary plus temperature shown in the bell-glass containing the uninjured objects, the other, usually bell-glass B, was filled with some substance to prevent the circulation of air, &c., and to render all the conditions as nearly equal as possible. Vessels containing KOH were always placed in the bell-glasses, and the objects supported above them. Formalin was employed to prevent the growth of fungi. The thermometers used were carefully compared, and found to be precisely alike and to agree with the standard thermometer.

EXPERIMENT 21.

400 grams potatoes in each bell-glass. Had been kept in warm room two days before using. Injured by cutting into quarters.

Duration of Experiment.		Temp. Bell-Glass A. °C.	Temp. Bell-Glass B. °C.	Difference.	Temperature outside. °C.
Uninjured June 1	4.00 p.m.	24.40	24·40	0.00	24·00
,, ,, 2	9.00 a.m.	24·15	24·15	0.00	23·70
Cut in quarters at 9.30 a.m.					
First day	12.30 p.m.	24·30	24·40	0·10	23·90
,,	3.30 ,,	24·25	24·60	0·35	23·60
,,	6.30 ,,	24·25	24·75	0·50	23·70
Second day	11.00 a.m.	24·30	25·10	0·80	23·70
,,	1.00 p m.	24·30	25·20	0·90	23·65
,,	4.00 p.m.	24·20	24·95	0·75	23·60
,,	6.30 ,,	24.25	24·80	0·65	23·70
Third day	9.00 a.m.	23·80	24·30	0·50	23·40
,,	12.00 noon	23·90	24·40	0·50	23·20
,,	3.00 p.m.	23·90	24·40	0·50	23·40
,,	6.00 ,,	24·00	24·55	0·55	23·60
Fourth day	9.00 a.m.	24·10	24·50	0·40	23·60
,,	4.00 p.m.	24·25	24·60	0·35	23·70
Fifth day	9.00 a.m.	24·30	24·65	0·35	23·80
,,	5.00 p.m.	24·30	24·50	0·20	24·00

EXPERIMENT 22.

600 grams potatoes in each bell-glass. In warm room two days before using. Washed in 1 °/$_o$ Formalin to sterilize. Injured as in Experiment 21.

Duration of Experiment.		Temp. in Bell-Glass A.	Temp. in Bell-Glass B.	Difference.	Temperature outside.
June 8	5.00 p.m.	25.80	25.80	0.00	25.30
,, 9	9.00 a.m.	25.80	25.80	0.00	25.35
	Cut in quarters at 9.10 a.m. Bell-glass B.				
First day	12.00 noon	26.10	26.20	0.10	25.60
,,	1.00 p.m.	26.20	26.50	0.30	25.65
,,	3.30 ,,	26.20	26.75	0.55	25.70
,,	4.30 ,,	26.25	27.00	0.75	25.60
,,	5.30 ,,	26.35	27.20	0.85	25.60
Second day	9.00 a.m.	26.50	27.45	0.95	25.65
,,	11.00 ,,	26.55	27.40	0.85	25.50
,,	1.00 p.m.	26.60	27.40	0.80	26.10
,,	4.00 ,,	26.50	27.30	0.80	26.10
Third day	9.00 a.m.	26.50	27.20	0.70	26.10
,,	3.30 p.m.	26.60	27.20	0.60	26.20
Fourth day	8.30 a.m.	26.60	27.00	0.40	26.10
Fifth day	8.30 ,,	26.40	26.60	0.20	26.00

EXPERIMENT 23.

450 grams radishes (*Raphanus sativus*) had been two days in warm room. Washed in 1 °/$_o$ Formalin. Injured by cutting longitudinally in quarters.

Duration of Experiment.		Temp. in Bell-Glass A.	Temp. in Bell-Glass B.	Difference.	Temperature outside.
June 18	9.00 a.m.	25.80	25.80	0.00	25.40
	Quartered at 9.15 a.m.				
First day	10.15 a.m.	25.80	26.00	0.20	25.40
,,	11.15 ,,	25.90	26.25	0.35	25.60
,,	12.15 p.m.	26.00	26.60	0.60	25.60
,,	[illegible].15 ,,	26.20	27.05	0.85	25.75
,,	4.15 ,,	26.20	27.15	0.95	25.75
,,	5.15 ,,	26.20	27.35	1.15	25.80
Second day	9.00 a.m.	26.20	26.80	0.60	25.70
,,	11.00 ,,	26.40	27.05	0.65	25.90
,,	1.00 p.m.	26.40	26.80	0.40	25.80

EXPERIMENT 24.

75 leaves of *Diervilla sp.* in each bell-glass. Weight, 50 grams. Kept in warm room fourteen hours before experiment.

Duration of Experiment.		Temp. in Bell-Glass A.	Temp. in Bell-Glass B.	Difference.	Temperature outside.
June 20	12.00 noon	26·20	26·20	0·00	26·10
Leaves slit longitudinally at 12·00.					
First day	1.00 p.m.	26·20	26·20	0·00	26·10
"	2.00 "	26·20	26·25	0·05	26·10
"	3.00 "	26·10	26·20	0·10	26·10
"	4.00 "	26·00	26·20	0·20	25·90
"	9.00 a.m.	25·80	25·90	0·10	25·90
"	12.00 noon	25·80	25·85	0·05	25·95

EXPERIMENT 25.

Leaves and small twigs of *Liriodendron tulipifera*. About 70 leaves, or 120 grams of leaves, in each bell-glass. Had been in laboratory one day before experiment was begun. At the end of the experiment the leaves, in bell-glass A, were found to be still quite fresh and turgid.

Duration of Experiment.		Temp. in Bell-Glass A.	Temp. in Bell-Glass B.	Difference.	Temperature outside.
June 24	5.00 p.m.	26·20	26·20	0·00	25·80
" 25	11.00 a.m.	26·20	26·20	0·00	25·80
Leaves slit longitudinally and petioles cut at 11.00 a.m.					
First day	1.00 p.m.	26·20	26·50	0·30	25·90
"	3.30 "	26·20	26·95	0·75	26·10
"	5.30 "	26·25	26·90	0·65	26·00
"	9.00 a.m.	26·50	26·90	0·40	25·80
"	3.30 p.m.	26·20	26·40	0·20	25·80
Second day	6.00 "	26·20	26·35	0·15	25·60
"	8.30 a.m.	26·15	26·20	0·05	25·60
"	12.00 noon	26·10	26·10	0·00	25·80

EXPERIMENT 26.

45 onion-bulbs (*Allium Cepa*)—500 grams—in each bell-glass. Had been in warm room two days before beginning of experiment. Washed objects with 1 °/₀ Formalin to sterilize them.

Duration of Experiment.		Temp. in Bell-Glass A.	Temp. in Bell-Glass B.	Difference.	Temperature outside.
June 30	6.00 p.m.	24·25	24·20	-·05	23·90
July 1	8.30 ,,	24·10	24·10	0·00	23·70
Onions in Bell-glass B cut into quarters at 8.45.					
First day	10.30 a.m.	24·15	24·25	0·10	23·80
,,	11.30 ,,	24·20	24·45	0·25	23·80
,,	12.00 noon	24·25	24·75	0·50	23·90
,,	12.30 p.m.	24·30	24·90	0·60	23·95
,,	1.00 ,,	24·50	25·20	0·70	24·00
,,	2.00 ,,	24·65	25·55	0·90	24·00
,,	3.30 ,,	24·55	25·90	1·35	24·10
,,	4.30 ,,	24·60	26·20	1·60	24·20
,,	5.30 ,,	24·60	26·40	1·80	24·20
,,	7.30 ,,	24·70	26·65	1·95	24·10
,,	9.30 ,,	24·80	27·20	2·40	24·05
Second day	8.00 a.m.	25·50	28·70	3·20	25·10
,,	10.00 ,,	25·50	28·80	3·30	25·10
,,	4.00 p.m.	25·50	28·30	2·80	25·20
Third day	9.00 a.m.	24·40	25·60	1·20	24·20
,,	4.00 p.m.	24·40	25·40	1·00	24·15
Fourth day	9.30 a.m.	24·40	25·00	0·60	24·00
,,	1.00 p.m.	24·40	24·85	0·45	24·10
,,	5.00 ,,	24·30	24·55	0·25	24·00
Fifth day	10.30 a.m.	24·40	24·50	0·10	24·20

EXPERIMENT 27.

500 grams small onion-bulbs (*Allium Cepa*), 3 cm. in diameter, in each bell-glass. In warm room three days before beginning of experiment. Washed with Formalin to sterilize.

Duration of Experiment.		Temp. in Bell-Glass A.	Temp. in Bell-Glass B.	Difference.	Temperature outside.
July 10	5.00 p.m.	26·40	26·40	0·00	25·20
July 11	9.00 a.m.	26·80	26·80	0·00	25·80
Cut onions in Bell-glass B in quarters 9.15 a.m.					
First day	11.30 a.m.	26·80	27·10	0·30	25·80
,,	1.30 p.m.	26·80	27·45	0·65	25·70
,,	3·30 ,,	26·85	28·20	1·35	25·70
,,	7·30 ,,	26·80	27·40	1·60	25·70
,,	9·30 ,,	26·80	27·70	1·90	25·80
Second day	10.00 a.m.	26·85	28·95	2·10	25·70
,,	12.00 noon	26·90	29·20	2·30	25·80
,,	6.00 p.m.	26·95	28·95	2·00	25·80
,,	9.00 ,,	27·00	28·80	1·80	25·90
Third day	8.00 a.m.	27·00	28·10	1·10	25·90
,,	12.00 noon	27·00	27·80	0·80	25·90
,,	6.00 p.m.	26·90	27·70	0·80	25·80
Fourth day	8.00 a.m.	26·80	27·30	0·50	25·70
,,	1.00 p.m.	26·80	27·25	0·45	25·80
,,	6.00 ,,	26·85	27·05	0·20	25·90
Fifth day	9.30 a.m.	26·90	27·00	0·10	25·80
The onions in Bell-glass B were removed and then replaced with coke. The temperature of the bell-glass with living onions above that of the other is shown below.					
Evening	5.00 p.m.	26·80	25·60	1·20	25·70
Next morning	9.00 a.m.	26·90	25·80	1·10	25·80

EXPERIMENT 28.

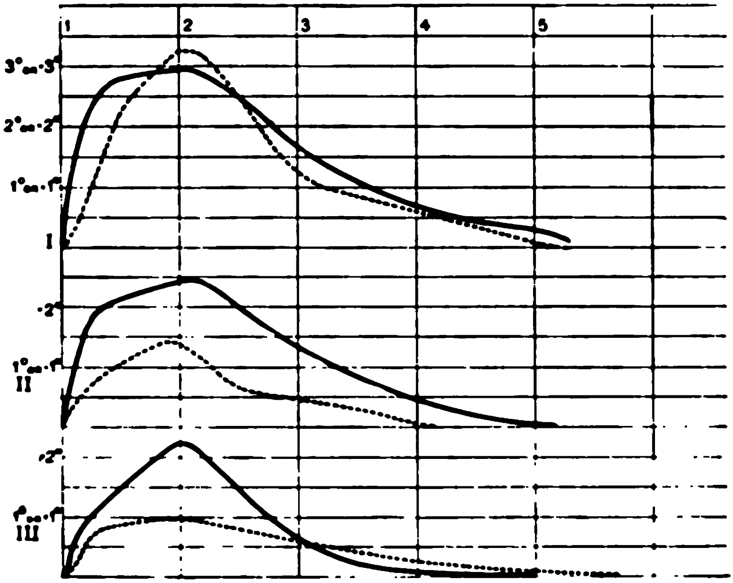
500 grams onion-bulbs (*Allium Cepa*), from 2–3 cm. in diameter, in each bell-glass. In warm room two days before using. Washed in 1 % Formalin to sterilize. Injured as in Experiment 27.

Duration of Experiment.		Temp. in Bell-Glass A.	Temp. in Bell-Glass B.	Difference.	Temperature outside.
July 14	5.00 p.m.	27·00	27·05	0·05	26·40
„ 15	8.00 a.m.	27·00	27·00	0·00	26·40
Cut onions in Bell-glass B in quarters 9.00 a.m.					
First day	10.45 a.m.	27·00	27·00	0·00	26·40
„	12.45 p.m.	27·00	27·30	0·30	26·45
„	2.45 „	27·00	27·60	0·60	26·35
„	4.45 „	27·10	27·95	0·85	26·40
„	6.45 „	27·10	28·20	1·10	26·40
„	8.45 „	27·10	28·70	1·60	26·40
„	10.45 „	27·10	29·10	2·00	26·30
Second day	10.45 a.m.	27·00	30·15	3·15	26·30
„	11.45 „	27·00	30·35	3·35	26·30
„	12.45 p.m.	27·00	30·40	3·40	26·30
„	2.45 „	27·00	30·40	3·40	26·40
„	4.45 „	27·05	30·05	3·00	2·40
„	6.45 „	27·05	30·05	3·00	26·40
Third day	8.45 a.m.	27·10	29·90	2·80	26·50
„	10.45 „	27·10	29·70	2·60	26·50
„	2.45 p.m.	27·05	29·35	2·30	26·30
„	6.45 „	27·00	29·00	2·00	26·20
Onions in Bell-glass B removed as in Exp. 27. Difference equals + temperature of Bell-glass A					
Evening	10 p.m.	27·00	26·25	0·75	26·20
Next morning		27·10	26·50	0·60	26·15

EXPERIMENT 29.

750 grams—20—small carrots (*Daucus Carota*) in each bell-glass. Had been three days in warm room. Washed with 1 °/₀ Formalin.

Duration of Experiment.		Temp. in Bell-Glass A. °C.	Temp. in Bell-Glass B. °C.	Difference. °C.	Temperature outside. °C.
July 6	7.30 a.m.	25·80	25·80	0·00	25·00
,,	11.00 ,,	25·80	25·80	0·00	25·00
Cut carrots in Bell-glass B in quarters.					
First day	3.00 p.m.	25·80	25·90	0·10	25·00
,,	5.00 ,,	25·70	26·20	0·50	24·90
,,	7.00 ,,	25·60	26·40	0·80	24·90
,,	8.00 a.m.	25·50	26·70	1·20	24·60
,,	10.00 ,,	25·40	26·65	1·25	24·60
,,	12.00 noon	25·40	26·75	1·35	24·70
,,	2.00 p.m.	25·40	26·40	1·00	24·50
Second day	4.00 ,,	25·35	26·20	0·85	24·50
,,	6.00 ,,	25·35	26·15	0·80	24·60
,,	8.00 ,,	25·40	26·00	0·60	24·70
,,	9.00 a.m.	25·45	26·05	0·60	24·80
,,	1.00 p.m.	25·30	25·80	0·50	24·80
Third day	5.00 ,,	25·20	25·60	0·40	24·60
,,	10.00 a.m.	25·40	25·55	0·15	24·70
Fourth day	4.00 p.m.	25·40	25·40	0·00	24·70
The carrots in Bell-glass B were removed and replaced by coke to compare temperature in Bell-glass A with surrounding air.					
Evening	7.00 p.m.	25·40	24·65	0·75	24·70
Next morning	10.00 a.m.	25·60	24·80	0·80	24·80



Woodcut 2.

Approximate temperature-curves of wounded plants. The solid lines are the curves obtained with the thermo-element, the dotted ones those from the bell-glass experiments. With the former the value of each division is 0·05° C., with the latter 0·5° C. The vertical lines show the days from time of injury.

I. Onion bulbs, Expts. 17 and 28. II. Carrots, Expts. 15 and 20.

III. Potatoes, Expts. 4 and 22.

Compare previous paper, l. c., Woodcut 3, p. 582.

Postscript. Since the MS. of this paper was sent to the printer, a *résumé*, by Prof. Pfeffer, of this and the previous article on respiration has appeared in Berichte der math. physisch. Classe d. Königl. Sächs. Gesell. zu Leipzig, Sitzung vom 27 Juli 1896.

A Contribution to our Knowledge of Lyginodendron.

BY

A. C. SEWARD, M.A., F.G.S.

With Plates V and VI.

AMONG a number of specimens in the Botanical Department of the British Museum referred to the genus *Dadoxylon*, I found one transverse section which had been cut from a thick stem possessing a large pith and a considerable development of secondary wood. An examination of the specimen revealed certain striking anatomical characters entirely at variance with those of *Dadoxylon* or *Cordaites*, but on the other hand indicative of a close affinity with the genus *Lyginodendron*. The section had been taken from an unusually fine piece of fossil wood partially enclosed in argillaceous limestone, having a length of 14 cm. and a breadth of 14 cm. A portion of the exposed surface of the specimen consists of smooth and waterworn wood; and where the fossil is enclosed by the surrounding matrix no trace of tissues external to the wood can be detected.

The wood measures 5·8 cm. in the thickest part, and very probably when the stem was living it attained a still greater breadth, as there is no proof that the outermost portion as shown in the fossil represents the extent of the original woody tissue. The large pith lying towards one side of the block

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measures 2·9 cm. in the widest part. Seen in longitudinal section, the pith shows here and there somewhat irregular and more or less transverse bands of dark-coloured tissue which, on superficial examination, resemble the horizontal disks of the pith of *Cordaites* (Pl. V, Fig. 2). Other sections of the same block were afterwards found in the Museum, and finally additional preparations were recognized in the Williamson Collection as having been obtained from the large piece of stem. From the entries in Professor Williamson's Catalogue of microscopic sections it was found that the material came from the Coal-Measures of Oldham, and had been contributed by Mr. Nield. The specimen when intact must have been one of the largest ever found in the English Coal-Measures in which the internal structure had been preserved.

In the fourth of the series of Memoirs on the 'Organization of the Fossil Plants of the Coal-Measures', Williamson writes as follows with regard to a specimen referred to *Lyginodendron*: 'I have obtained from Mr. Nield one magnificent axis in which the woody cylinder and its contained medulla has been at least eighteen inches in circumference. The thickness of the wall of this vascular cylinder has been at least 2½ inches; and, since the specimen is weathered and water-worn, it may have been of even larger dimensions.' In a foot-note the specimen is said to have been 'found in a watercourse intersecting the Lower Coal-Measures at a locality near Oldham known as Har Culver (Higher Culvert)[1].' In the recent memoir on *Lyginodendron* and *Heterangium* by Williamson and Scott, the authors refer more than once to Nield's large specimen. Discussing the size of *Lyginodendron* stems, these authors write: 'The largest *undoubted* stem of *Lyginodendron* which we possess attained a diameter of about 4 cm. We leave out of consideration for the present both Mr. Nield's specimen and the cortical impressions. These must have belonged to stems of enormously greater size, but we cannot absolutely prove their identity with *Lyginodendron*[1].' On page 742 of the same work we find the following account of the large Oldham fossil:—

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[1] Phil. Trans., 1873, p. 386. A radial section of this specimen is figured in Plate XXIII, Fig. 9 (No. 1183 in the Williamson Collection).

'There remains the large specimen showing structure, received from Mr. Nield, and referred to in Memoir IV. p. 386. The specimen includes the pith and a portion of the wide zone of secondary wood. Sections in the three directions have been cut and clearly exhibit the structure so far as it is preserved. The diameter of the pith is 3·3 x 2·3 cm. The maximum radial thickness of the secondary wood is 5·8 cm., but we cannot be certain that its whole thickness is preserved. Assuming, however, that we have the whole thickness of the wood, the radius of the stem up to the cambium would have been over 7 cm., and its diameter over 14 cm. We cannot tell what was the diameter of the whole stem, for we know nothing of the cortex. No authentic specimen of *Lyginodendron* which we have seen, however, has secondary wood of a greater thickness than about 6 mm.; so, if we judge by this dimension, the stem in question must have been nearly ten times as large as that of any undoubted *Lyginodendron* in the Williamson Collection. Unfortunately, the only structure preserved is that of the secondary wood. Its general anatomy is identical with that of certain specimens of *Lyginodendron*. The tracheids are smaller than is usual in *Lyginodendron*, but not smaller than in some undoubted stems of that plant. The radial section shows the muriform rays and the pits on the walls of the tracheids, which, in so far as their preservation allows of comparison, agree very well with those of our plant. The pith is completely disorganized, and no trace of the primary wood can be recognized. There is, however, a narrow incomplete zone of internal secondary wood, distinct from the rest, at the margin of the pith which recalls the anomalous medullary tissue sometimes found in *Lyginodendron*. Although the parenchyma of the pith has perished, the cavity contains clusters of dark brown cells, which are much like the sclerotic nests characteristic of the pith of *Lyginodendron*. On the whole, until some other fossil has been found which agrees better with this doubtful stem, we think there is a presumption that it really belonged to a *Lyginodendron*, or to some plant of the same type of structure[1].'

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[1] Phil. Trans., Vol. 186, 1895, B, pp. 738, 706.

An examination of additional sections cut from Mr. Nield's specimen has furnished a few more facts as to the anatomical structure, and the main object of the present paper is to amplify and illustrate by means of a few figures the description given by Williamson and Scott. The material on which the following account is based consists of (1) the water-worn block mentioned by Williamson in 1873, and now in the Botanical Department of the British Museum; (2) twelve sections in the Botanical Department; and (3) the following sections in the Williamson Collection[2] (British Museum), viz. Nos. 1131, 1132, 1133, 1183, 1184, 1185. All the sections have been cut from the single specimen supplied to Professor Williamson by Mr. Nield. In Pl. V, Fig. 1, is represented a photographic reproduction of a transverse section. The diameter of the pith and wood has already been given. The large pith is seen to be partially occupied by irregular patches of tissue, which in longitudinal section (Fig. 2) assume the form of broken transverse bands. The inner margin of the secondary wood consists of small dark bands separated by lighter radially-elongated spaces. This is more clearly seen in Fig. 6 and in the lower part of Fig. 3; the lighter and broader patches were originally occupied by medullary-ray tissue, and the darker lines represent the internal limits of rows of secondary tracheids. In Fig. 1, and more clearly in Fig. 6, the continuity of the inner margin of the wood is interrupted at three points, *t*, *t'*, and *t''*, which mark the position of outgoing groups of xylem-elements, probably leaf-trace bundles[3].

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[1] Phil. Trans., Vol. 186 (1895) B, p. 742.

[2] There are also two small sections in a collection of fossil plants in the Botanical Museum, Cambridge.

[3] These groups of tracheids in passing through the secondary xylem of the stem

The dark concentric lines seen in a transverse section of the wood, and especially well marked on the right-hand side of the photograph (Fig. 1), are for the most part due to the occurrence of lines of narrower tracheids, locally developed in response to some influence which was much too irregular to be the result of seasonal changes. In Fig. 2 the wood and pith are shown in radial section: the mottled appearance of the former is due to the numerous and large medullary rays which form a characteristic feature of the stem. In Figs. 3 and 4 the character of the wood is more clearly seen; the lower end of Fig. 3 is in the immediate neighbourhood of the inner limit of the wood, and here the tracheids are fewer in number and farther apart than in the more external portion of the wood. The spaces between the narrow and curved rows of tracheids correspond to those seen less distinctly in Figs 1 and 6: in this region of the stem the medullary-ray-parenchyma has usually disappeared.

The xylem is entirely composed of tracheids with reticulately-pitted radial walls (Fig. 5); the nature of the pitting on the tracheids which form the innermost limit of the wood cannot be clearly seen owing to imperfect preservation. The xylem-elements are arranged in radial rows varying in breadth from one row of tracheids to bands composed of eight rows. In tracing bands of tracheids towards the periphery of the stem the number of elements composing any one band in a tangential direction is found to vary considerably, as the result of the intercalation of new rows or of a fusion between adjacent bands. The medullary rays consist of radially elongated parenchymatous cells of one to ten rows in breadth, the average breadth of a ray being nearly equal to that of the tracheidal bands. The broad medullary rays give to the wood the characteristic appearance noticed in the description of Fig. 2. Towards the inner margin of this wood the medullary-ray cells are usually absent, but in places where they have been preserved they are found to be tangentially elongated

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[1] [illegible] course, very similar to that of the leaf trace [illegible]

between the widely separate bands of xylem. In Figs. 7 and 8 the wood is shown in tangential and radial section; in the former the polygonal cells of the medullary rays are seen to occupy long and narrow meshes, usually with pointed ends, in a framework of tracheids. No trace of bordered pits has been noticed in the tangential walls of the wood-elements. In radial section (Fig. 8) the reticulate pitting is found to be fairly well preserved, but the borders of the pits are for the most part imperfect (Fig. 5); the medullary-ray-cells present the characteristic appearance of radially-elongated parenchymatous cells, with here and there dark patches suggesting the contents of secretory cells. Seen under a higher power, the radial walls of many of the tracheids are found to be traversed by sets of parallel and obliquely running lines. It is difficult in many cases to determine how far such parallel markings on the xylem-elements of fossil plants are the result of cleavage in the infiltrated mineral substance, or to what extent they are the expression of some histological feature. In the tracheids of fossil coniferous wood we frequently find delicate and regular lines agreeing very closely in appearance with the striation characters of recent tracheids, and probably due to the emphasizing by ferment-action and decay of the original striations in the substance of the tracheid-walls. In the present instance it is probable that the lines referred to are in some measure due to the original striation of the tracheids.

The structures hitherto described in detail constitute the secondary centrifugally-developed wood. If the rows of tracheids in the secondary wood be traced to their inner termination, they are found to taper off to an acute apex, or to merge into a small group of tracheids in which the radial arrangement is either entirely absent or indistinctly marked. As already pointed out, the medullary-ray-cells have usually been destroyed in this region, and the spaces separating the inner terminations of the tracheid bands are often bounded by a fairly regular line, concave towards the pith; this marks the limit of decay, and recalls to some extent the appearance presented by the broad medullary rays of a Calamite stem (Pl. VI, Fig. 10). In the parenchymatous tissue internal to the tapered ends of the tracheid-rows, and to a less extent in the broad medullary-ray-tissues, there are found several patches of black substance which, no doubt, mark the position of secretory cells. Internal to the centrifugal xylem there extends round the periphery of the pith a series of short rows of tracheids, often arranged in groups with a fan-like disposition and with the apex facing the centrifugal wood. These series of tracheids are separated here and there by distinct medullary rays. This inner and much smaller zone of secondary tissue constitutes the centripetally-developed xylem. In Pl. V, Fig. 6, the faint line immediately internal to the centrifugal wood, and surrounding the pith, marks the position of the centripetally-developed tracheids; these are also semi-diagrammatically shown in Pl. VI, Fig. 14*x*. In Pl. VI, Fig. 10, the inner limit of the centrifugal wood is shown on either side of a bay forming a break in the outer ring of xylem, and probably due to an outgoing leaf-trace-bundle. In Pl. VI, Fig. 16, a fairly well preserved portion of the centripetal xylem is figured; it consists of rows of tracheids, sometimes as many as sixteen tracheids occurring in a single radial series, separated by bands of imperfectly preserved medullary-ray-parenchyma. The radial walls of these tracheids appear to have the same form of pitting as occurs in the centrifugally-developed xylem. Internally the centripetal ring is succeeded by very imperfectly preserved tissue, but in some places it can be readily seen that a merismatic layer existed on the pith-side of the inner ring of wood. The crushed and partially disorganized tissue internal to the wood contains numerous dark-coloured patches of tissue (*s*) and an abundance of secretory cells. The former, of which one (*s*) is shown in Fig. 16, internal to the centripetal wood, are nests of sclerous cells, of which several occur in the region of the pith (Fig. 10). In a longitudinal section the remains of phloem-tissues are found internal to the centripetal xylem, and in many places the tissues have been torn, apparently along the line of cambium, which gave rise to the centripetal xylem. Internal to the phloem a patch of medullary parenchyma is preserved, containing a sclerous nest and several black patches of secretory cell-contents.

Reserving for further consideration the nature and relations of the centripetal and centrifugal xylem, we may pass on to describe the pith. In Plate V, Fig. 2, the lighter portions in the pith mark the spots where the tissues have been destroyed and their place taken by mineral substance; the dark patches represent parenchymatous tissue containing numerous sclerous nests, which appear as dark dots in the photograph, and secretory cells. On examining transverse and longitudinal sections of the pith under a low magnifying power, one sees distinct signs of active merismatic division as expressed by the arrangement of parenchymatous cells in regular serial rows. Some of the darker portions in Fig. 2 consist of secondary parenchyma developed from a zone of meristem situated on the external limits of the darker-coloured and rounded outlines of the tissue-patches. In Fig. 14 a portion of the pith is represented, very slightly enlarged; immediately internal to the centripetal wood there are patches of parenchymatous tissue containing secretory cells and three sclerous nests; internal to this, and separated from it by an intervening band of mineral matrix, we have a mass of parenchyma of which a considerable portion is made up of a series of regular radiating rows of elements formed by cambial activity, the cambium being situated at the inner edge of the secondary tissue (*c*). This secondary medullary parenchyma presents the same appearance in longitudinal as in transverse sections.

In describing the occurrence of anomalous xylem in the pith of *Lyginodendron Oldhamium*, Williamson and Scott[1] point out that in some cases the cambium gives rise to secondary parenchyma instead of xylem and phloem. The secondary parenchyma in the pith of *Lyginodendron robustum* is, on the other hand, a product of a meristem internal to the centripetal wood. A tissue of precisely similar appearance occurs in a specimen of *Lyginodendron Oldhamium* which Williamson figured in his Memoir IV (1873), Pl. XXII, Fig. 4[1]. The dark patches of tissue internal to the wood consist of radially-disposed rows of parenchyma developed by a zone of cambium, and exactly corresponding in structure with the masses of tissue in the pith of *Lyginodendron robustum*. It is conceivable that the secondary parenchyma may be of a corky nature, and analogous to the periderm-tissue described in the pith of *Stangeria paradoxa*[2] and in some stems of *Bennettites*[3].

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[1] Loc. cit., p. 722. See also the reference on p. 723 to 'Some specially complicated forms of anomalous tissue in the pith of *Lyginodendron Oldhamium*.'

There remains to be considered the nature of the xylem-bands. Unfortunately the tissues between the outer edge of the centripetal xylem and the inner edge of the centrifugal xylem are very imperfectly preserved. In a few places, however, the two bands of wood are seen to be in direct continuity. The tracheids of the two zones of wood are frequently separated by a gap in the tissues; and, as previously noticed, the bands of centrifugal wood when traced to their inner termination often pass into groups of tracheids, which do not show anything of the regular arrangement characteristic of secondary xylem. In Plate VI, Fig. 12, and in Figs. 9 and 16, the characteristic appearance of the imperfectly mineralized tissue between the two sets of xylem-elements is represented. In one or two places between the centripetal and centrifugal wood, traces have been observed of tracheids showing indications of spiral thickening; it is possible that these may be the protoxylem-elements of the primary wood, but the preservation is hardly such as to justify any very decided statement.

This brings us to a comparison with the structure of the xylem as described by Williamson and Scott in *Lyginodendron Oldhamium* (Binney). In that species the centrifugally-developed secondary xylem agrees in the main very closely with that of the stem described above. On the inner side of the centrifugal wood there occur in *Lyginodendron Oldhamium* groups of primary xylem ; each group represents a leaf-trace-bundle and possesses a well-defined mesarch structure, the position of the primary xylem-elements being recognized by the presence of the narrow spirally-thickened protoxylem-tracheids, and the larger scalariform tracheids, in addition to the reticulately marked elements[1]. In some examples of *Lyginodendron Oldhamium* an inner zone of centripetal xylem has been described, probably identical with that in Nield's specimen. This inner xylem is described by Williamson and Scott as 'anomalous wood,' and compared with similar structures in certain recent Dicotyledons[2]. In attempting to establish the precise affinities of the stem under discussion it is extremely important to decide, as far as possible, whether or not there are any traces of primary xylem like that of *Lyginodendron Oldhamium*. Owing to the imperfect state of preservation in that part of the stem where the primary xylem should occur, it is very difficult to give a decided and satisfactory opinion as to the original existence of such primary groups as occur in the previously recorded examples of the genus.

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[1] No. 1143. Cf. also 1114, 1118 and other specimens in the Williamson Collection.

[2] Solms-Laubach, Bot. Zeit. Jahrg. 48, p. 11.

[3] Capellini and Solms-Laubach, I Tronchi di Bennettitee (Mem. R. Accad. Sci. Inst. Bologna), Vol. ii, 1891, p. 48, Plate V, Figs. 2, 5.

It is, in the first place, conceivable that the primary xylem-elements have become disorganized during the growth and increase in thickness of the stem. This possibility necessitates considerable caution in relying on negative evidence. On the other hand there are certain appearances presented by sections of Nield's stem which, without absolutely proving the existence of primary xylem-tracheids, render it probable that traces of such groups have been partially preserved. There are often found groups of tracheids, or occasionally single isolated tracheids, between the radially-disposed bands of centrifugal and centripetal wood. These, it may be, are portions of primary xylem-strands. The unfortunate imperfection in the mineralization of the tissues in this region, renders it impossible to make use of such characters as the nature of the pitting as a distinguishing feature of the primary xylem-tracheids. In *Lyginodendron Oldhamium*, for example, the scalariform character of the tracheids affords a convenient distinguishing feature of the primary xylem in longitudinal section. In one or two longitudinal sections imperfectly preserved tracheids have been detected of which the faint traces of pitting suggest the scalariform type, such as occurs in the primary xylem of *Lyginodendron Oldhamium*, but the evidence is insufficient to be cited as absolute proof.

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[1] See the figures and descriptions in Williamson and Scott's paper.

[2] Williamson and Scott, loc. cit., pp. 722, 723.

In Plate V, Fig. 6, at *t* and *t′*, is shown a group of tracheids in contact laterally and distally with the main mass of centrifugal xylem; these groups are probably leaf-trace-bundles on their way to the surface of the stem. In Plate VI, Fig. 15, a single leaf-trace is seen under a higher magnifying power; the dark lines indicate the direction of the rows of tracheids, and the centripetal xylem is represented at x^2. It is not possible to recognize at the proximal end of the outgoing leaf-trace-bundle any definite indication of primary xylem-elements: the space may be in part the result of tearing of the tissues in the immediate neighbourhood of the primary tracheids. In another section showing a leaf-trace-bundle in a similar position to that represented in Fig. 15, there are groups of fairly thick-walled elements lying about internal to the fan-shaped trace; these may be portions of the primary xylem. At the distal end of a somewhat obliquely-cut leaf-trace as seen in a transverse section of the stem there is a mass of narrow and radially-elongated parenchymatous cells. In tangential sections of the stem the leaf-trace-bundles are seen passing through the centrifugal xylem, and in their immediate neighbourhood the tracheids of the wood exhibit extraordinary contortions. In Fig. 11 some of the strongly curved centrifugal tracheids are shown in connexion with an outgoing leaf-trace[1]. One of the small sections (1185) in the Williamson Collection shows the strongly marked curvatures of the tracheids remarkably well.

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[1] A very similar appearance is presented by a tangential section of a [illegible].

It has been pointed out by Williamson and Scott that in *Lyginodendron Oldhamium* the internal cambium may arise 'partly from the parenchyma of the primary xylem, so that some of the tracheae belonging to the latter have been carried inwards into the pith[1].' By this means the original position of the primary xylem-elements would be altered, and their subsequent recognition rendered more difficult. This possibility should be borne in mind in connexion with the apparent absence of primary xylem-strands in Nield's stem.

To compare, briefly, *Lyginodendron Oldhamium* and the larger stem described above, as regards the centrifugal wood, there is on the whole a very close agreement; but a careful comparison of sections from the British Museum stem with numerous examples of *Lyginodendron Oldhamium* confirms the opinion expressed by Williamson and Scott, that in Nield's specimen the tracheids are somewhat smaller than in the undoubted examples of *Lyginodendron*. In *Lyginodendron Oldhamium* the medullary rays are usually narrower, and smaller in proportion to the breadth of the tracheid-bands than in the larger stem. The anomalous wood described by Williamson and Scott in some examples of *Lyginodendron* is precisely similar in structure to the ring of centripetal wood in Nield's stem. In some examples of *Lyginodendron Oldhamium* the tissues of the pith present the same regular radial arrangement of parenchymatous cells as in the large stem. The sclerous nests and secretory sacs are practically identical in distribution and appearance in *Lyginodendron Oldhamium* and Nield's stem.

In transverse sections of *Lyginodendron Oldhamium* one occasionally finds that the secondary centrifugal wood and the primary xylem assume the appearance of an almost continuous band without any obvious division between them;

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stem, figured by Renault in a 'Notice sur les Calamariées.' Autun, 1895, Plate IV, Fig. 6.

[1] Loc. cit., p. 723.

presenting, in fact, an appearance very similar to that already noticed in certain parts of the large stem[1]. Sections of *Lyginodendron Oldhamium* occasionally show an apparent continuity between the centrifugal and centripetal xylem-bands, the primary xylem being sometimes disturbed and much less obvious than in other parts of the section.

In the remarks on two sections (Nos. 1129 and 1130) written in the catalogue of the Williamson Collection, the great breadth of the medullary rays is pointed out; the secondary wood in the specimens is very similar to that in Nield's stem, but the tracheids in the former are slightly wider. The same sections show also the curving of the inner ends of the tracheid-bands, as in the large stem; the groups of primary xylem in these sections present a crushed appearance and are separated by wide intervals. The general appearance of the sections is exceedingly close to that of Nield's plant[2]. The comparison of several specimens of *Lyginodendron Oldhamium* with the stem under discussion, as regards the manner of occurrence of the primary xylem, renders it probable that the absence of any direct *proof* of the existence in the latter of undoubted primary elements by no means negatives the idea that the plant originally possessed primary xylem-strands similar to those of the former species. A tangential section through the wood of *Lyginodendron Oldhamium* presents the appearance of longer and narrower medullary rays than those represented in Pl. V, Fig. 7; but although there is a fairly constant difference in this respect, by examining a sufficient number of sections it is possible to match fairly closely the various structures in the two sets of specimens.

The general conclusion arrived at may be briefly stated as follows. The large stem which forms the subject of the present paper, should be placed in the genus *Lyginodendron*, but is in all probability not specifically identical with *Lyginodendron Oldhamium* (Binney). No actual proof of the occurrence of primary xylem-tracheids has been discovered, but such evidence as is available is favourable rather than opposed to the original existence of such primary strands. As a convenient designation of Nield's large stem, of which the diameter must have been very considerably greater than in any previously known specimen of the genus, we may adopt the name *Lyginodendron robustum*.

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[1] e.g. No. 1130 in the Williamson Collection.

[2] Williamson noticed the close resemblance in the wood of the two sections 1129 and 1130 to that of Nield's specimen. In his catalogue he speaks of the wood and medullary rays of these two sections as practically proving the identity of Nield's specimen and *Lyginodendron Oldhamium*.

With reference to the numerous examples referred to *Lyginodendron Oldhamium*, Williamson and Scott write: 'All the forms with which we are concerned may be provisionally referred to the same species, or rather type, *Lyginodendron Oldhamium*.' As these words imply, it may be possible with more complete knowledge to recognize additional specific types among the numerous examples of the genus. As regards a comparison with recent plants, the most striking resemblance is that between the centrifugal wood of *Lyginodendron robustum* and the wood of Cycadean stems. An examination of sections of the secondary wood of *Cycas*, *Stangeria*, and *Macrozamia* reveals the closest possible resemblance with *Lyginodendron*. In the stems of *Cycas* and *Macrozamia*, however, the wood does not form a broad continuous band as in *Lyginodendron robustum*, but consists of concentric zones developed from successive meristems. The cycadean nature of the centrifugal wood of *Lyginodendron Oldhamium* has been previously pointed out by Williamson and Scott[1] and others[2], but the much larger size of the stem of *Lyginodendron robustum* makes the resemblance still more striking than in the much smaller form, *Lyginodendron Oldhamium*.

In 1879 Renault described a new type of a Palaeozoic plant from the well-known silicified beds of Autun, to which he gave the name *Cycadoxylon Fremyi*[3]. A comparison of the figures illustrating the structure of this Autun specimen with those of *Lyginodendron robustum* brings out a striking similarity as regards the structure of the centrifugal wood. The tangential section figured in Pl. V, Fig. 7, of the present paper is very similar to the corresponding section of *Cycadoxylon* (Renault, Pl. XIV, Fig. 12). There is also a close agreement between the transverse and radial sections of the wood of the two plants (cf. Renault, Pl. XIV, Figs. 10 and 11, and *Lyginodendron robustum*, Figs. 4, 8, 10, 16). More recently M. Renault has supplemented his earlier account of *Cycadoxylon* by additional figures and a further description published in an important work on the coal-field of Autun and Épinac.

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[1] Loc. cit., p. 767.

[2] Solms-Laubach, Fossil Botany (1891), p. 361.

[3] Structure comparée de quelques tiges de la Flore Carbonifère (Nouv. Arch. Mus. Paris, Vol. ii [2], 1879, p. 283. See also Cours de botanique fossile, Vol. i. (1880), p. 74, Plate XI.

The following description of the structure of the wood as seen in a transverse section of *Cycadoxylon Fremyi* illustrates the most striking characters: 'Le système ligneux est composé d'un cylindre extérieur continu d'arcs ligneux dispersés dans la moelle en nombre variable. Le cylindre extérieur est formé de séries rayonnantes de trachéides, ponctuées, à accroissement centrifuge; les bandes ligneuses intérieures sont constituées par des séries rayonnantes semblables, mais dont l'accroissement est centripète'[1]. Renault compares this genus, of which *Cycadoxylon Fremyi* is the only example, with *Ptychoxylon* and *Medullosa*. The centrifugal and centripetal bands of xylem are separated from one another by parenchymatous tissue, and there is no indication of any continuity between them in a radial direction, nor are there any groups of primary xylem internal to the centrifugal wood such as occur in *Lyginodendron Oldhamium*. Since writing this paragraph I have had an opportunity, through the kindness of M. Renault, of examining the sections of *Cycadoxylon*. In one specimen a small group of primary xylem was clearly shown.

In Fig. 56 (p. 309) of the Flore fossile d'Autun et d'Épinac, a portion of a transverse section of *Cycadoxylon*

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[1] Bassin Houiller d'Autun et d'Épinac. Flore fossile, Pt II, p. 307. I am indebted to M. Renault for kindly forwarding me a proof of that part of his forthcoming work which deals with *Cycadoxylon*. The text of this work has now appeared. January, 1897.

Fremyi is represented, showing an internal and narrow band of centripetal xylem, composed of tracheid-rows separated by broad medullary rays, and external to this a narrow zone of parenchyma, followed by a broader zone of centrifugal xylem. The broad band of the latter wood consists of rows of tracheids and broad medullary rays practically identical with the corresponding xylem of *Lyginodendron robustum*, the centripetal wood is also precisely alike in the two plants. The absence of any cortical tissues in *Lyginodendron robustum* prevents any comparison with the cortex of *Cycadoxylon*. 'Gum-canals' are fairly numerous in the tissues of the latter genus, as in *Lyginodendron*.

In the pith of *Cycadoxylon* there appear to be no sclerous nests, and in the cortical tissues there are none of the radially-disposed bands of sclerenchyma characteristic of *Lyginodendron Oldhamium*. As at present defined, the genus *Cycadoxylon* is no doubt distinct from *Lyginodendron*; the resemblance, which is indeed particularly close between the centrifugal and centripetal wood of the two plants, *Cycadoxylon Fremyi* and *Lyginodendron robustum*, is one based on secondary structures. There is no evidence of any similar agreement as regards the more important primary structures[1].

In 1878[2] Williamson figured and described three sections of a fragment of wood from the volcanic ash of Arran which he named *Lyginodendron anomalum*. A re-examination of the sections by Williamson and Scott led them to regard the specimen as having 'nothing in common with the genus *Lyginodendron*' but as rather comparable with Renault's *Cycadoxylon*[3]. In transverse section the Arran fragment shows bands of reticulately pitted tracheids and broad medullary rays, with some of the tracheids occasionally cut across in an obliquely longitudinal direction. In tangential section we have an irregular network of tracheids with the large meshes occupied by groups of polygonal parenchymatous cells. The tangentially-cut medullary rays assume the form of oval, circular, or oblong groups of cells, bounded by the extremely sinuous and contorted tracheids. The chief difference between the tangential section of *Lyginodendron anomalum* and *Lyginodendron robustum* consists in the broader and shorter medullary rays of the former, and the more irregular and sinuous course of the tracheids. In a section through the centrifugal wood of *Lyginodendron robustum* in the neighbourhood of an outgoing leaf-trace, the tracheids are similarly contorted to those in *Lyginodendron anomalum*. In a tranverse section of the former passing through a leaf-trace-bundle, as shown in Pl. V, Fig. 6, *t* and *t'*, and Pl. VI, Fig. 15, the broad distal end of the trace is followed by long and narrow parenchymatous cells, which have accompanied the tracheids from the inner edge of the wood. The general appearance of such a section is almost identical with that of a transverse section of *Lyginodendron anomalum*. Such a comparison suggests the possibility that the shorter and broader medullary rays and the more irregular course of the tracheids may not represent the normal character of the stem from which the Arran fragment was obtained, but that these appearances may be the result of some disturbing influence in the secondary wood. The resemblance between *Lyginodendron robustum* and *Lyginodendron anomalum* as regards the structure of the wood and the form of the medullary rays, which is specially striking in the wood of the former species where the normal form of the rays is modified by the bending of the tracheids to a leaf-trace-bundle, points to the possibility of the two forms being closely allied to one another. The Arran fragment is much too small to allow of precise identification, but the structure of the wood renders it extremely probable that in *Lyginodendron anomalum*, Will., we have a small piece of a stem which possessed secondary wood of the cycadean type very similar in structure to that of *Lyginodendron*

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[1] The existence of a group of primary xylem, referred to in a previous footnote, makes the agreement still closer.

[2] Phil. Trans., 1878 (Memoir IX of Williamson), p. 252, Plate XXV, Figs. 90–92. The specimen from which the sections were obtained is in the Geological Museum of the University of Glasgow.

[3] Williamson and Scott, loc. cit., p. 770 (footnote).

robustum and *Cycadoxylon Fremyi*. In the absence of more complete specimens, it is impossible to decide which of these generic names shall be adopted [1]; but the Arran plant may, I believe, be regarded as closely allied to *Lyginodendron Oldhamium* and *Cycadoxylon Fremyi*. The figure of a transverse section of *Lyginodendron anomalum* given by Williamson in his Memoir IX, Pl. XXV, Fig. 90, does not bear so close a resemblance to *Lyginodendron robustum* as is apparent in another section recently cut from the same specimen and now added to the Williamson Collection. In this section the broad medullary rays and bands of tracheids are more regular in their arrangement, and the section as a whole exhibits a striking similarity to that of *Lyginodendron robustum*. A small portion of this section is shown in Pl. VI, Fig. 13; in some of the bands of tracheids the individual elements are occasionally cut across obliquely, but on the whole the structure is like that shown in the figure.

Without attempting to give a specific diagnosis, we may briefly summarize the more important characteristics of the specimen for which the name *Lyginodendron robustum* is suggested, and which has hitherto been spoken of as 'Nield's specimen.' The centrifugal wood is composed of reticulately-pitted tracheids somewhat smaller in diameter than those of *Lyginodendron Oldhamium*; the medullary rays are rather broader, and there is a more regular continuous zone of centripetal xylem than in *Lyginodendron Oldhamium*. In the latter plant this internal development of xylem is found to vary considerably in amount in different regions of the same transverse section. In the transverse section 1885 E (Williamson Collection), the 'anomalous wood' is well shown, but internal to one of the primary xylem-strands it is absent.

This particular group of xylem is seen to be in direct continuity with the external centrifugal wood in such a manner as to closely resemble the appearance presented by some of the sections of *Lyginodendron robustum*. Williamson and Scott do not attach the slightest importance to the anomalous medullary cambium as a specific character; they point out that 'among stems, which are perfectly similar in other respects, some show it and some do not, while in those that possess this anomaly, the degree in which it is developed is most variable[1].'

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[1] In addition to the sections in the Williamson Cabinet (No. 1208 and three others), there is a somewhat larger tangential section, apparently from the same specimen, in the Binney Collection (Cambridge). The original specimen is in the Hunterian Museum, Glasgow University.

Numerous sclerous nests and secretory sacs occur in the pith of *Lyginodendron robustum*, and the latter are also present between the centrifugal and centripetal wood, and in the medullary-ray-tissue. An extended examination of several sections leads to the conclusion that Williamson and Scott's opinion that Nield's specimen 'really belonged to a *Lyginodendron*, or to some plant of the same type of structure,' is no doubt correct. *Lyginodendron robustum* represents by far the largest stem of the *Lyginodendron* type so far recorded, and the considerable thickness of the secondary xylem renders more obvious the close correspondence with the wood of recent cycadean stems, than is apparent in the much smaller stems of *Lyginodendron Oldhamium*.

In certain genera of fossil plants, one of the most prominent characteristics is the very close resemblance of their centrifugal xylem to the wood of recent Cycads. *Lyginodendron*, *Heterangium* and *Cycadoxylon* afford three examples of such agreement, and other genera might be mentioned. It is not unusual to discover specimens of plant-fragments in which the secondary xylem-characters are clearly preserved, but from which it is impossible to formulate a complete diagnosis.

It is often impossible to rely on the structure of the secondary wood alone as a means of specific or even generic identification; but the nature of the xylem-elements, the structure and form of the medullary rays, usually enable us to refer a specimen to a definite family or group of plants. In dealing with coniferous wood we are able to adopt certain recognized generic designations, such as *Araucarioxylon*, *Pityoxylon*, and others, which, as somewhat comprehensive terms, are extremely useful in systematic work. The term *Cycadoxylon* proposed by Renault, if extended in its application, might serve as a general generic designation for plants possessing secondary xylem closely resembling that of recent Cycads. Such a term would be particularly useful in dealing with imperfect material; while the more complete data obtained from better specimens would enable us to make use of generic designations of a less comprehensive meaning.

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[1] Loc. cit., p. 723. Examples of *Lyginodendron Oldhamium* showing an unequal development of centripetal wood are figured by Williamson (Mem. XVII. Phil. Trans., 1890, Plate XIII, Fig. 3) and by Williamson and Scott (Phil. Trans., 1895, Plate XXIII, Fig. 8). Both sections were cut from the same specimen; also sections 1143 and 1894 F.

In his important Memoir on the comparative structure of certain Coal-Measure stems, Renault includes the three genera *Cycadoxylon*, *Colpoxylon*, and *Medullosa* in the group Cycadoxyleae; and as a characteristic of the group he mentions that the genera in question have lost the centripetal wood in their stems, while retaining it in their leaves[1]. In the more recent, and fuller, account of *Cycadoxylon*, Renault figures and describes well-marked bands of centripetal wood, thus necessitating either a modification of the original definition of the Cycadoxyleae, or a removal of *Cycadoxylon* from that group of plants in which the stem has no internal centripetal wood.

If *Cycadoxylon* were adopted in a wider sense than as defined by Renault, it would be the more appropriate genus to which to refer *Lyginodendron anomalum*. In the case of *Lyginodendron robustum* alone it might perhaps be better to make use of this generic designation; but the presumptive evidence in favour of a generic identity with *Lyginodendron Oldhamium* is so strong, that there appear to be sufficient grounds for the choice of the more restricted genus *Lyginodendron*. Without attempting to institute a comparison with the various Palaeozoic genera possessing cycadean characters, it would seem that *Lyginodendron robustum*, *Lyginodendron Oldhamium*, *Lyginodendron anomalum*, and *Cycadoxylon Fremyi*, possess such characters in common as distinctly point to a close relationship.

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[1] Nouv. Arch. Museum, Paris, 1879, p. 282.

Our incomplete knowledge of the last named species, as compared with our much more complete acquaintance with *Lyginodendron Oldhamium*, renders it unprofitable to press the comparison very far; but it is probable that we may be able, with more extended knowledge, to classify many of the Palaeozoic types into more definite groups founded on the true relationship as expressed by the possession of cycadean characters.

The structure of *Medullosa gigas*, Ren., as described in the Flore fossile d'Autun et d'Épinac, presents an exceedingly close agreement with that of *Lyginodendron* (Flor. foss., Pl. LXXI). A more complete comparison of the various Palaeozoic plants possessing cycadean affinities must, however, be deferred.

My thanks are due to Mr. George Murray, Keeper of the Botanical Department in the British Museum, for permission to have several sections prepared from material in his charge; to Mr. Gepp, of the British Museum, I am indebted for the photographs reproduced in Plate V; and to Dr. D. H. Scott for valuable criticisms and suggestions.

EXPLANATION OF FIGURES IN PLATES V AND VI.

Illustrating Mr. Seward's paper on *Lyginodendron*.

All the sections have been prepared from the same specimen of *Lyginodendron robustum*, sp. nov., and are in the Botanical Department of the British Museum.

PLATE V.

Fig. 1. Transverse section of the stem, showing the thick centrifugal wood with apparent annual rings, and the tissues of the pith, with secondary parenchymatous tissue at *p*.

Fig. 2 Longitudinal section (radial) showing the characteristic appearance of the wood as seen in radial section, and the imperfectly preserved tissues of the large pith. (Figs. 102 are from photographs by Mr. Gepp, and represent approximately the natural size of the sections.)

Fig. 3. Portion of a transverse section of the centrifugal wood, with the broad medullary rays especially distinct at the lower end of the figure (from a photograph by Mr. Hayles of the Cavendish Laboratory, Cambridge). × 17.

Fig. 4. Portion of the wood of Fig. 3 more highly magnified, showing the broad medullary rays. × 52.

Fig. 5. Radial wall of a tracheid of the centrifugal wood. × 320.

Fig. 6. The inner edge of the wood and the pith-parenchyma: t, t', t'' = leaf-trace-bundles, x^2 = centripetal wood, s = sclerous nests (natural size).

Figs. 7, 8. Tangential and radial longitudinal sections through the centrifugal wood. × 52.

PLATE VI.

Fig. 9. Transverse section showing the inner termination of the centrifugal wood (x^1), and the imperfectly preserved bands of centripetal tracheids (x^2), and medullary rays. × 52.

Fig. 10. Transverse section showing the characteristic tapered rows of tracheids at the internal limit of the centrifugal wood, separated by spaces left on decay of the broad medullary rays: x^2 = centripetal xylem, s = sclerous nests (slightly enlarged).

Fig. 11. Tangential longitudinal section passing through the centrifugal wood in the immediate neighbourhood of a leaf-trace-bundle. The figure represents only a portion of the outgoing trace.

Fig. 12. Transverse section showing the inner ends of centrifugal tracheids and the outer limit of centripetal tracheids. × 52.

Fig. 13. *Lyginodendron anomalum.* Transverse section. × 52.

Fig. 14. *Lyginodendron robustum.* Slightly enlarged sketch of the inner edge of the xylem and the peripheral tissues of the pith; p = radially-arranged secondary parenchyma, c = meristem; x^1 = centrifugal, and x^2 = centripetal xylem; s = sclerous nests.

Fig. 15. Transverse section including a leaf-trace-bundle cut across somewhat obliquely, * = space at proximal end of the leaf-trace (slightly enlarged).

Fig. 16. Transverse section to show the fairly well preserved centripetal xylem (x^2). × 52.

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Fig. 1.

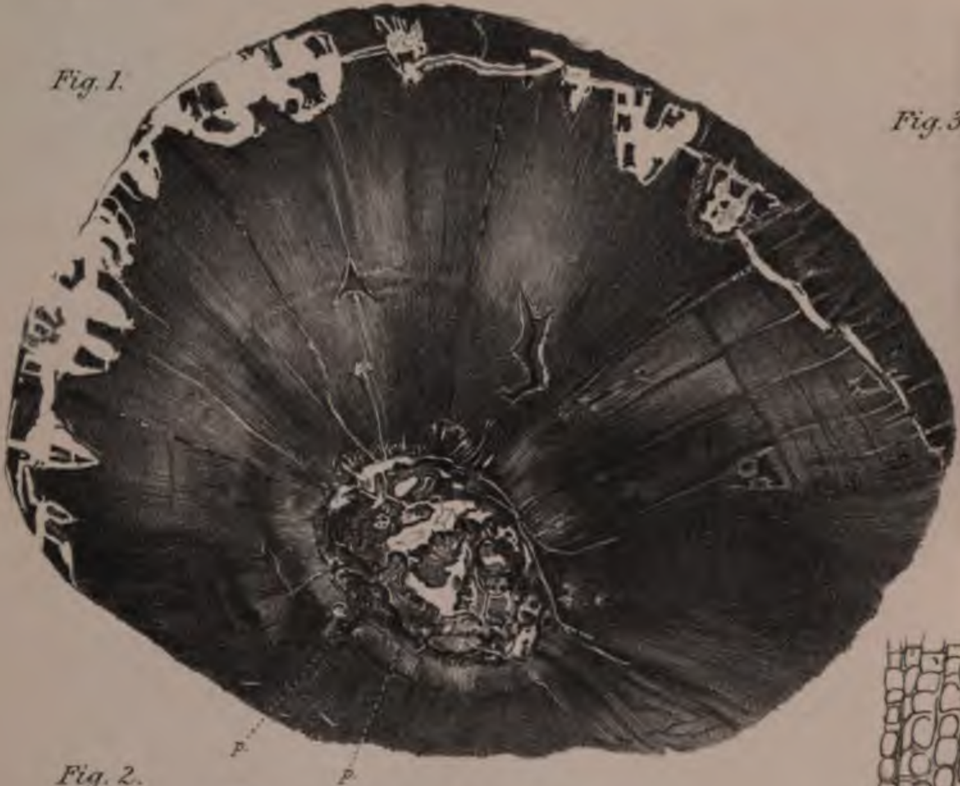


Fig. 3.

Fig. 2.

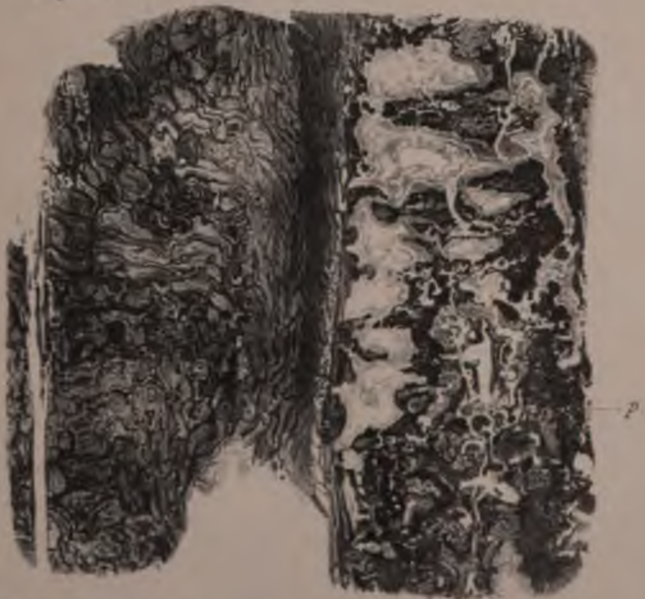


Fig. 4.

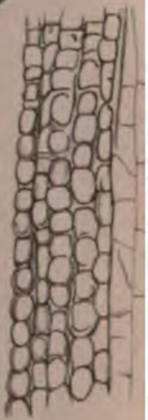
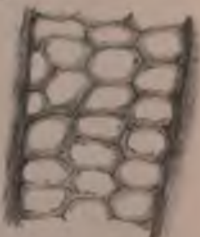


Fig. 5.



Figs 1 & 2, from photo. by A. Gepp.
Figs 3-5, A. C. Seward del.

SEWARD. — LYGINODENDRON.

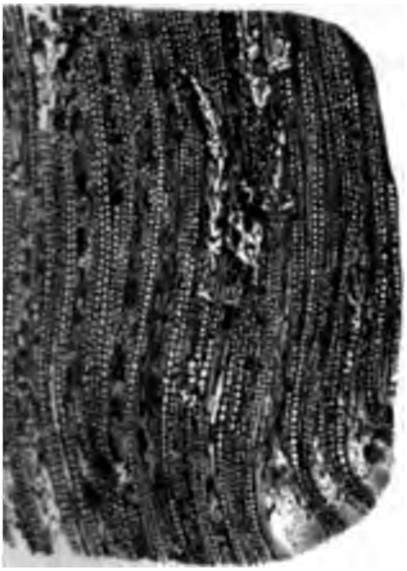
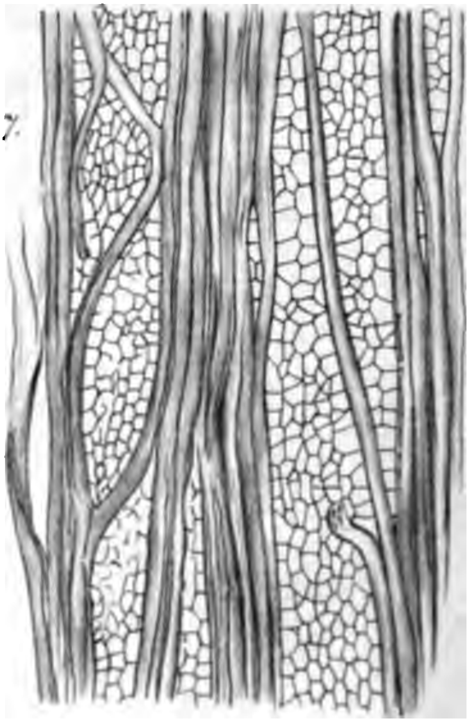


Fig. 7.



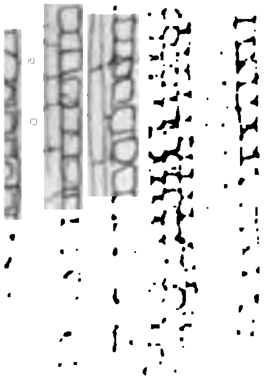


Fig. 8

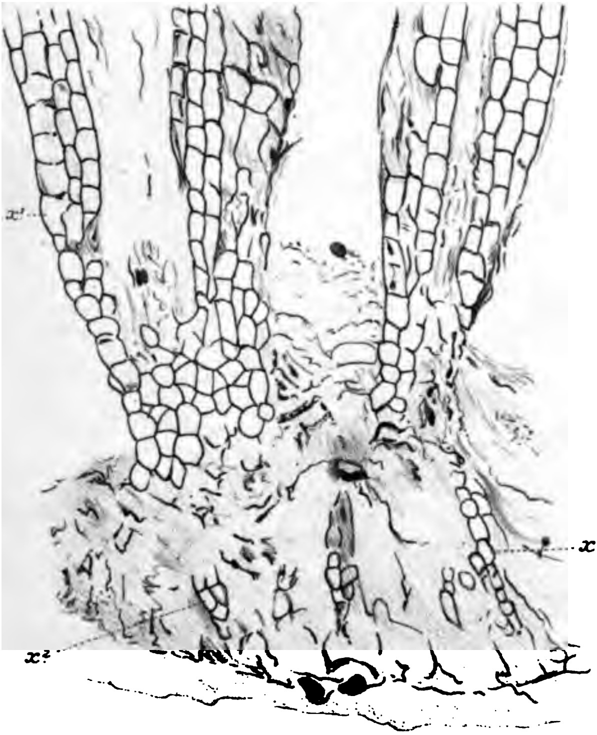


Fig. 9.

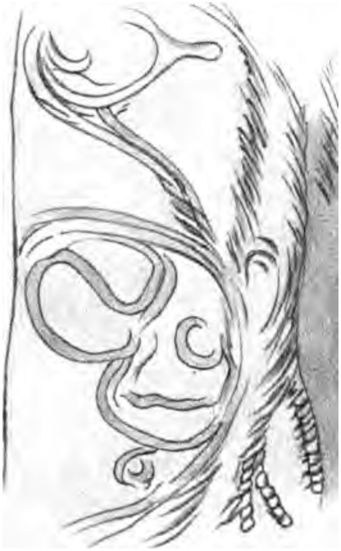


Fig. 11.

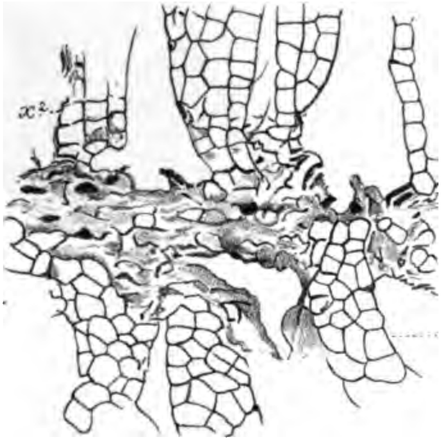


Fig. 12.

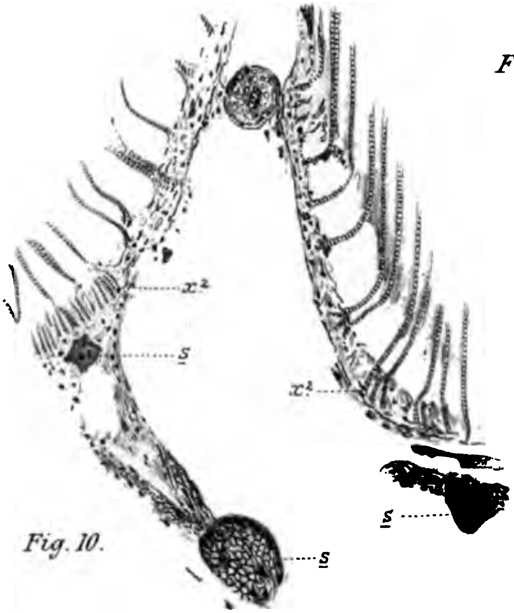


Fig. 10.

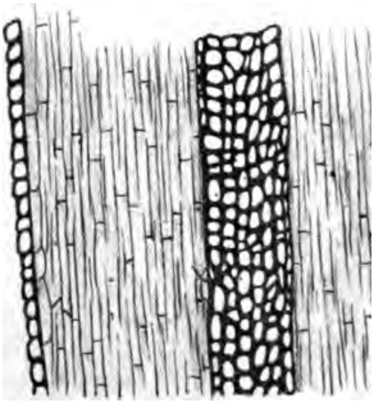


Fig. 13.

A.C.Seward del

SEWARD. — LYGINODENDRON.

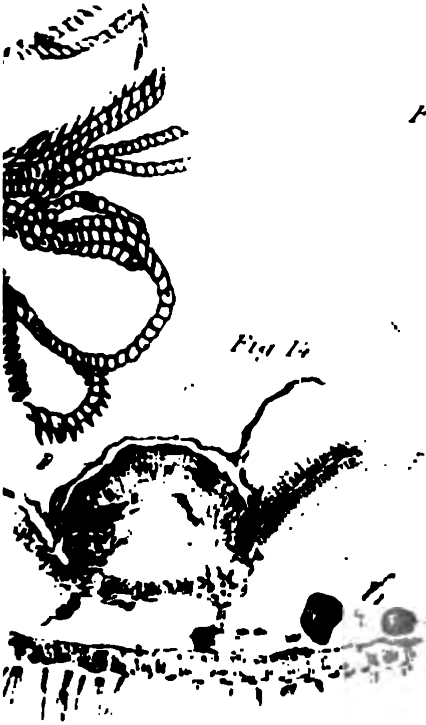


Fig. 14.

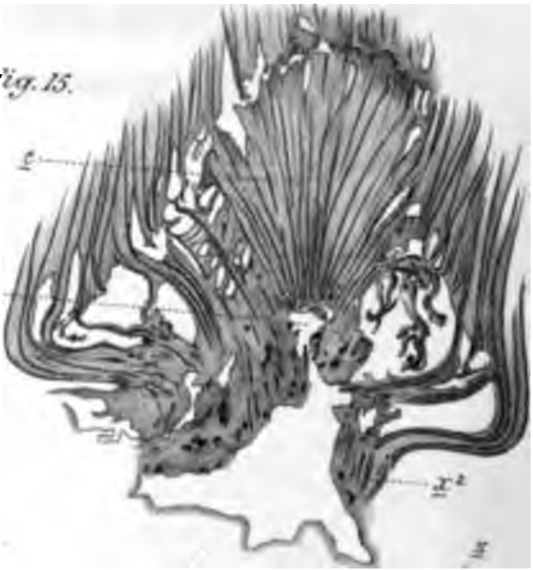


Fig. 15.

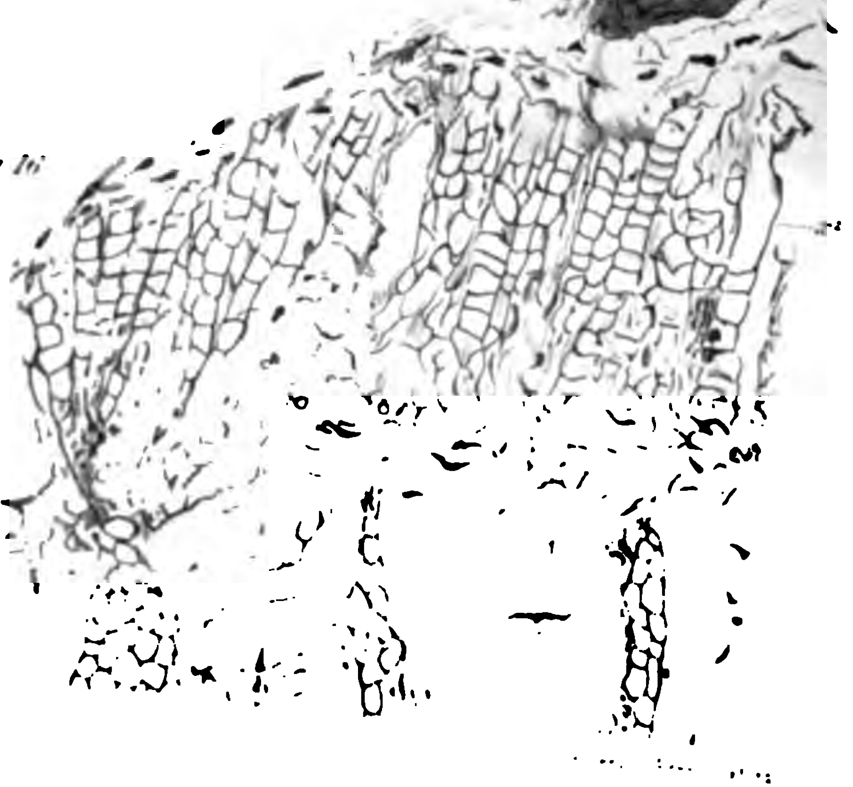


Fig. 16.

On some Species of the Genus Urophlyctis[1].

BY

P. MAGNUS (Berlin).

With Plates VII and VIII.

ON March 16, 1882, at the meeting of the Botanical Section of the 'Schlesische Gesellschaft fur vaterländische Cultur,' J. Schroeter described the development of the old *Physoderma pulposum*, Wallr.[2] He pointed out that it produces large swarm-sporangia which rest superficially upon the epidermis of species of *Chenopodium*, and send forth fascicles of rhizoids into the cells. It also develops resting-sporangia by the conjugation of two cells of similar form inside the living tissue of the host. One of these cells empties its contents into the other, which then grows into the resting-sporangium. Upon these characters he based the genus *Urophlyctis* in the 'Kryptogamen Flora von Schlesien,' v. III. part I. p. 196 (1886). There he also added a second species under the name of *U. major* (*U. majus* in the text) which does not form swarm-sporangia.

In 1888 I described a third species, parasitic on *Carum Carvi*, which I named *Urophlyctis Kriegeriana*. It is con-

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[1] [illegible] 1896.

[2] [illegible] 1882 [illegible]

[3] [illegible] Berlin, p. 100

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fined to the epidermal cells, which, owing to their being attacked in this way, swell out, together with the adjacent cells, into a pearl-shaped gall, having a depressed umbo at the apex. The apical orifice leads into the much-enlarged epidermal cell which contains the Fungus. This species of *Urophlyctis*, which does not extend beyond the epidermal cell, forms resting-sporangia by the conjugation of two cells derived from different mycelial threads. This I described in the paper cited.

In the second edition of Rabenhorst's 'Kryptogamen-Flora von Deutschland, Oesterreich und der Schweiz,' vol. II. Pilze, Part IV, Phycomyceten, p. 131, Alfred Fischer unites the genera *Cladochytrium* Nowak., *Physoderma* Wallr., and *Urophlyctis* Schroet., into one genus *Cladochytrium*, and in it he places the three above-mentioned species. As to the formation of the resting-spores in this enlarged genus *Cladochytrium* he says (p. 132): 'Die Dauersporen entstehen entweder an Stelle der Sporangien aus der inhaltsreichen Zelle der zweizelligen Anschwellungen und tragen dann ebenfalls eine kleinere, leere Anhangszelle; oder terminal an kurzen, von den Sammelzellen ausgehenden unverzweigten Fäden, deren Enden anschwellen und, ohne sich wieder zu theilen, zur Dauerspore werden.' Fischer does not therefore admit the process of conjugation from which the resting-spores of *Urophlyctis* originate, but considers the antheridium to be that cell of the two-celled swellings which is less rich in protoplasm.

Without any mention of Fischer's opinion, however, Schroeter retains the genera *Physoderma*, *Cladochytrium*, and *Urophlyctis* in Engler and Prantl's 'Natürlichen Pflanzenfamilien,' I. Theil, 1. Abtheilung, pp. 81 and 86, and refers the last-named genus to his tribe Oochytriaceae. Possibly at the time when Schroeter wrote, Fischer's paper had not yet been published, and nevertheless he quotes Fischer's 'Phycomycetes'[1] amongst the more important literature on the Chytridiaceae. It may be that the last number was issued during the revision of the proofs.

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[1] In Rabenhorst's Krypt.-Fl. Deut., I. Bd., iv$^{\text{te}}$ Abtheil. Leipzig, 1892. Lieferungen 45–47.

I can confirm Schroeter's statements concerning the formation of the resting-spores of the oosporangia, having repeatedly examined *Urophlyctis Kriegeriana*, *U. pulposa*, and also a third species belonging to this genus.

As mentioned above, *U. Kriegeriana* presents the appearance of pearl-like galls on the surface of the stems, leaves, and floral parts of *Carum Carvi*. Neighbouring galls often fuse more or less completely, thereby forming smaller or larger hyaline punctate crusts, especially on the surface of the stems (Fig. 1). Every gall has externally at its apex a more or less depressed umbo (Figs. 1 and 2), which leads into a very large cell occupying the centre of the gall. *U. Kriegeriana* occurs only within this cavity. The inner membrane of this enlarged central cell always reaches the base of the umbo, and is at that point exposed; but with that exception the cavity is surrounded on all sides by a wall consisting of many layers of cells. The membrane at the free apex is traversed by a mycelial thread which dilates immediately below into a vesicle, from which, in young galls, hyphae originate (Figs. 3, 5 and 9). These hyphae form swellings which grow into conjugating cells (Figs. 6, 7), and hyphae of the same kind are attached to many points of the cell-wall. They either immediately produce the conjugating cells or, more rarely, they first form a swelling from which delicate hyphae arise (Fig. 6), and these form the conjugating cells. Conjugation takes place as described by Schroeter. The cells, which conjugate by means of a connecting channel, always originate from distinct hyphae. At first the conjugating cells differ but slightly in size, one of them, however, soon enlarges considerably, whilst the other remains small, and its contents pass through the connecting channel into the larger cell (Figs. 12, 13).

It is noteworthy that the male cells giving off their contents always spring from one distinct set of hyphae; so that we have here male plantlets developing at long intervals male cells which conjugate with female cells originating from other hyphae (Figs. 10 and 13). The hyphae often branch, especially from the male cells (Figs. 10 and 11).

As Schroeter has already described in the case of *Urophlyctis pulposa*, the receptive and enlarging cell separates also, in the present instance, from the parent hypha. Therefore I have not been able to determine whether a single hypha produces several receptive or female cells.

The female cell enlarges, becomes filled with dense protoplasm containing oil-globules, and develops a thick brown cell-wall. On the side where conjugation with the male cell takes place it remains flat and depressed in the centre. The process of conjugation can be most clearly observed, as Schroeter has done in the case of *U. pulposa*; and I believe that Fischer was led to his conclusions rather from descriptions, and the statements made by Büsgen as to the formation of resting-sporangia in *Physoderma*, than from actual personal investigation of the subject.

From the structure of the gall, as described, it is most probable that the germs of the parasite enter an epidermal cell, which in consequence swells considerably and causes frequent divisions in the surrounding epidermal cells. The particular cell attacked by *U. Kriegeriana* becomes the central cavity of the gall, whilst the surrounding epidermal cells undergo repeated subdivision and form a wall around it consisting of several layers of cells, leaving at the apex a crater-like opening.

As before stated, *U. Kriegeriana* grows only within the enlarged cavity of the gall, and never extends beyond it. The development of the wall of the central cell corresponds with this. The wall thickens considerably, almost attaining the width of the neighbouring cell-layer of the envelope of the gall. It never exhibits the sieve-like perforations or the grating-like appearance which De Bary and Schroeter have described in *U. pulposa*. The strong imperforate membrane is not traversed by the delicate hyphae of the Fungus, and the parasite remains confined to its enlarged host-cell.

I have never observed the formation of other propagative organs, especially the development of zoosporangia, although I have often directed my attention to this point. The formation of zoosporangia seems to be wanting here, as in the case of Schroeter's *U. major*, or it may, perhaps, be restricted to the germination of the oospores.

Urophlyctis Kriegeriana is very common on *Carum Carvi* throughout Europe. Besides Saxony and Bohemia, the only habitats known to me up to 1888, it has since been found in Thuringia by F. Thomas[1], and Herr E. Ule has discovered it near Coburg, according to a specimen communicated to me. G. de Niessl has collected it near Schwarzkirchen in Moravia, and distributed it under the name of *Protomyces macrosporus*, Ung., in Rabenhorst's Fungi Europaei, No. 1100. Herr Friederich Stolz gathered it in the Gschnitz valley and in the Stubai valley, Tyrol; and Blytt has found it in Norway[2].

I am able, however, to record its occurrence not only on *Carum Carvi*, but also on *Pimpinella Saxifraga*. The Fungus which W. Voss has indicated on *Pimpinella Saxifraga* as *Synchytrium aureum*, Schroet., in the first part of his Mycologia Carniolica (in 'Mittheilungen des Musealvereins für Krain,' 1889), p. 17, is my *Urophlyctis Kriegeriana*, as I have proved by a specimen communicated to me by Herr W. Voss himself. Perhaps the parasite also occurs on other Umbellifers[3].

Another species of *Urophlyctis* I knew to be the cause of a serious disease of the Beetroot in Algiers. L. Trabut reported[4] on a disease observed on the thick primary roots of *Beta vulgaris*, var. *rapacea*, at Rouiba, near Algiers. It consists of thick fleshy botryoid swellings contracted at the base. Trabut proved these to be due to the presence of a Fungus which he named *Entyloma leproideum*, and described and figured it afterwards in the 'Revue Générale de Botanique,' T. vi (1894), p. 409, under the name of *Oedomyces leproides*, Saccardo having founded upon it the genus *Oedomyces*. Subsequently Saccardo and Mattirolo published an elaborate paper on the subject in Malpighia anno X (1895). Trabut, Saccardo and Mattirolo state that the Fungus occurs in cysts provided with a strong membrane (quasi cellulas giganteas). The cysts are scattered irregularly through the swellings, and the spores are produced from evanescent hyphae by acrogenous and intercalary abstriction. Saccardo distinguished it from *Entyloma*, mainly 'cystis vel singularibus et forma subhemisphaerica sporarum.' Trabut describes the spores thus: 'De forme sphérique déprimée avec pédicelle très court, inséré sur une éminence au centre de la face déprimée. . .'

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[1] Mittheilungen des botanischen Vereins für Gesammtthüringen, Bd. viii, 1889.

[2] Bidrag til Kundskaben om Norges Soparter, iv, p. 25, in: Christiania Videnskabs-Selskabs Forhandlinger, 1896, No. 6.

[3] [illegible] gathered by J. Bornmüller in Persia, 1892–93, and sent to me for investigation [illegible] *Urophlyctis* [illegible] Bornmüller [illegible] Kerman, on Mount Kuh-tagh-Ali, near the city of Kerman [illegible] No. 4402 of J. Bornmüller, Iter Persico-turcicum 1892–93.

[4] Comptes Rendus de l'Académie des Sciences de Paris, June 4, 1894.

These authors refer the Fungus to the Ustilagineae. Saccardo and Mattirolo support this view by pointing to the formation of the cysts within the tissue of the root of the host-plant, to the new growth caused by the parasite, to the acrogenous and intercalary origin of the spores, and finally to the structure and colour of the latter. Ed. Prillieux, in his work, 'Maladies des plantes agricoles et des arbres fruitiers et forestiers, causées par des parasites végétaux,' Tome I (Paris, 1895), pp. 193-197, refers to this disease and assigns the Fungus to the Ustilagineae.

Thanks to the kindness of Professor O. Mattirolo, who sent me a specimen in alcohol, I have been able to examine this Beetroot parasite. It proves to be a *Urophlyctis*, and must therefore bear the name *U. leproides* (Trab.), P. Magn. The cysts have a wall of exactly the same thickness as the central cell of the gall of *U. Kriegeriana*, and the parasite lives also exclusively within the cysts, as the authors above cited have shown. According to these authors the cysts are embedded singly in the parenchyma of the swellings, and originate from the hypertrophy of the cells attacked by the parasite. I cannot quite confirm this. I found rather that many cysts are connected with each other by narrow tubes originating from the cysts (Fig. 17). Each cyst throws out pointed processes which penetrate the parenchyma, and then at intervals widen out again into fresh cysts; each new cyst does the same and so on (Figs. 17–22). It often happens that many such tubular processes spring from one cyst; they may be short or long, wide or narrow; and they may frequently be followed a long way through the tissue. The tubes are provided with the same kind of thick membrane as the cysts. The observation of young processes and small cysts shows that the protoplasm affected by the parasite enters them, and that this protoplasm leads the way for the *Urophlyctis* that follows at a later period. In any case I have not succeeded in tracing the hyphae of the Fungus in the protoplasm of the youngest swellings. The parasite, however, soon follows, and entering these swellings often forms new spores within their wider portions (Fig. 18).

It is only in the narrowest portions of the channels, as shown, for instance, in Figs. 22 and 23, that the formation of spores is suppressed. The majority of the cysts of one swelling are therefore connected with each other, or rather they are excrescences of a single cyst pervading the parenchyma of the swelling.

The only difference between this growth and that of the gall of *Urophlyctis Kriegeriana* is that the development of the cell attacked by the latter parasite, and consequently also that of the gall, is limited. On the other hand, in the case of *U. leproides*, the cell attacked by the Fungus grows without limit; it pervades the parenchyma, the frequent divisions of which are the cause of the cell's growth into the new tissue, while it reacts on the parenchyma as a stimulus for still further divisions. Thus the large botryoid swellings on the upper part of the root of *Beta vulgaris*, var. *rapacea*, make the disease very conspicuous.

The wall of the infected cell is also in this case very thick, and it never possesses sieve-like perforations as in *U. pulposa* and *U. major*. The formation of spores takes place in exactly the same manner as in *U. Kriegeriana.* In this species there are also certain hyphae which produce only male cells, and often branch from these cells (Figs. 24, 28–30). It is remarkable, as I have observed, that the male cells are sometimes divided by septa (Figs. 32, 33). They conjugate by means of a narrow connecting channel through which the protoplasm of the male cell passes into the female (Figs. 24, 28, 29). The conjugating cells are alike, or differ only slightly in size at first (Figs. 28 and 32); but the receiving-cell soon enlarges considerably and separates from the mother-thread, the remains of which are only seldom perceptible (Figs. 29 and 33 *r*). Thus it would appear that conjugation always takes place on the side opposite the stalk. This conjugating side remains also flattened and usually somewhat depressed in the centre, as the authors quoted above have described and figured, and eventually a strong brown membrane is formed. No swarm-sporangia are known: it is, however, quite possible that they exist, as so far only the cysts of the inner tissue of old galls have been examined, not the surface of young galls. The specimen I examined did not show any zoosporangia.

The case is very different in *Urophlyctis pulposa*, the development of which has already been described by Schroeter in its more essential features. The parenchymatous cells infected by the parasite enlarge considerably, but at the same time the wall is perforated, owing to the action of the parasite, by local gelatinization, whereby their protoplasm, together with that of the parasite living in it, passes into the surrounding cells (Figs. 14–16), causing in this case also considerable enlargement (Fig. 14). Thus the parasite wanders through the perforations of the wall caused by local gelatinization of the membrane from one cell to another, and all the infected parenchymatous cells of an infected area represent a continuous system of cavities pervaded by the parasite, and often intermixed with unchanged small parenchymatous cells (Fig. 14).

Schroeter, in Engler and Prantl, Natürl. Pflanz., I. Theil, 1. Abtheil., p. 86, also refers his *Physoderma Butomi* to the genus *Urophlyctis* without giving any reasons for so doing. Should it actually belong to this genus, and form resting-sporangia—in contradiction to the statements by Büsgen—by conjugation, then it would represent a *Urophlyctis* which wanders from cell to cell by means of its mycelium, and does not cause the host-cells to increase in size. In future years I hope to extend my investigations to such species of *Physoderma.*

In every case it is of great interest to discover the different ways in which the various species of *Urophlyctis* affect the host-cell, and the tissue of the plants on which they are parasitic.

The figures which accompany this paper have been drawn from nature by Dr. Paul Roeseler in my presence. To Dr. Stapf I am much indebted for his kind assistance in translating this paper.

EXPLANATION OF FIGURES IN PLATES VII AND VIII.

Illustrating Professor Magnus's paper on *Urophlyctis.*

Figs. 1–13. *Urophlyctis Kriegeriana,* Magnus, on *Carum Carvi* from Prossen in Saxony.

Fig. 1. Transverse section of the stem with several confluent galls. × 15. The galls are cut in the tangential or median planes; only the latter show the umbo (*u*). × 15.

Fig. 2. Transverse section of the stem with a single gall cut through the apex (*u*). × 106.

Figs. 3–5. Longitudinal sections of the apex (*u*) of the galls. The short mycelial threads that have penetrated the membrane of the infected cell are shown enlarging into vesicles from which originate long mycelial threads. × 390.

Fig. 6. A single mycelial thread attached to the cell-wall. It shows a vesicular enlargement from which spring hyphae that give rise to conjugating cells.

Fig. 7. Mycelial threads attached to the cell-wall. These have formed conjugating cells. × 390.

Fig. 8. Mycelial threads attached to the cell-wall. × 390.

Fig. 9. Apex of the host-cell (*u*) showing the vesicle from which originate the mycelial threads. One mycelial thread is shown attached to the cell-wall.

Figs. 10–13. Conjugating cells. The male conjugating cells are modified from special mycelial threads with long internodes, which frequently branch out from the male cells. They conjugate with the female cells through narrow fertilization-channels. x 390.

Figs. 14–16. *Urophlyctis pulposa* (Wallr.), Schroet., on *Chenopodium.*

Fig. 14. Large parenchymatous cells of *Chenopodium rubrum* (Karlsbad, Bohemia) in which the parasite grows; next to unchanged parenchymatous cells which remain small. The walls of the neighbouring infected cells are pierced in a sieve-like manner, and the mycelial threads may be seen passing through the pores from one cell to the other. At *a* a young parenchymatous cell is shown, into which, in the young state, the mycelial threads of *Urophlyctis pulposa* hav entered through the pores in the cell-wall. The cell is beginning to swell; the mycelial threads have as yet formed no conjugating cells. x 420.

Figs. 15–16. Cells in which *Urophlyctis pulposa* has developed, showing the sieve-like perforated walls. x 207.

Figs. 17–31. *Urophlyctis leproides* (Trab.) P. Magn., on *Beta vulgaris*, var. *rapacea*, from Rouiba in Algiers.

Figs. 17–23. Transverse sections of the galls showing the cysts.

Fig. 17 shows several cysts connected together by channels which arise from the cysts. The latter are shown also in Figs. 19–21. Figs. 22 and 23 exhibit the separate long connecting channels of the cysts of the galls. Fig. 18 shows these in part cut in transverse section. (Figs. 17–20, x 111; Figs. 18, 19, and 22, x 162; Figs. 21–23, x 420.)

Figs. 24–32. Conjugating cells formed from mycelial threads.

In Figs. 24, 28, and 29 are shown the threads which give rise only to male fertilizing cells with long intermediately segmented cells, which branch out from the fertilizing cells. Figs. 25–27 show the fertilization-channels. Figs. 29 and 33 show at *r* the remains of the carrying threads of the female receptive cells.

In Figs. 32 and 33 the male cells have become divided. x 420.

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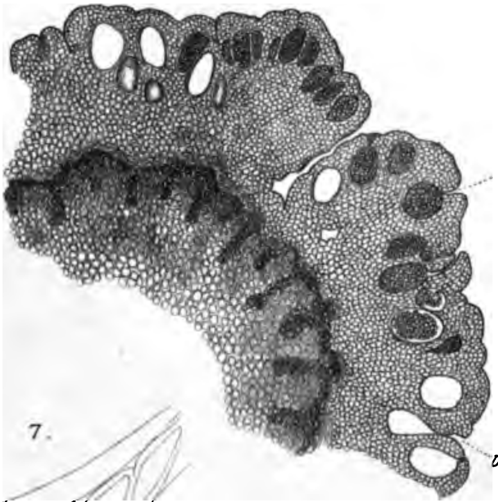

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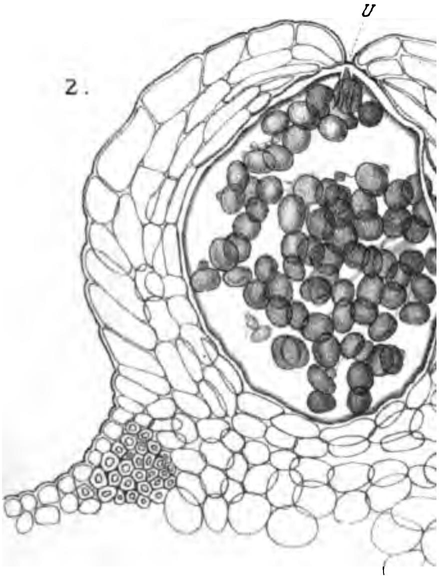

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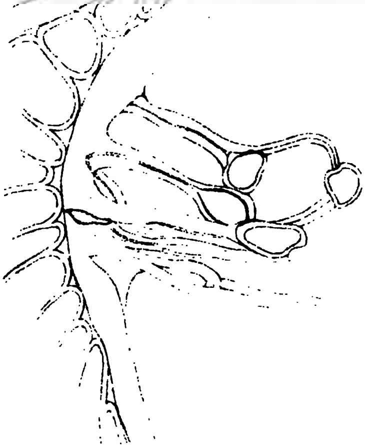

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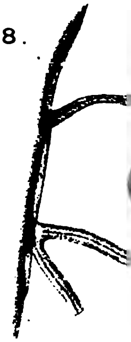

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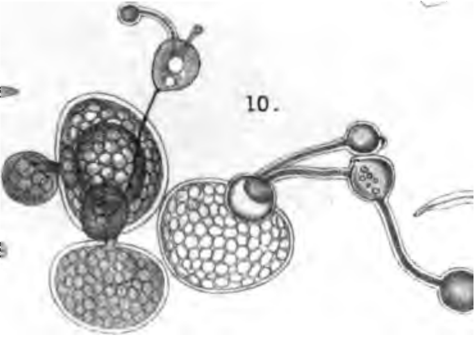

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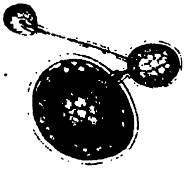

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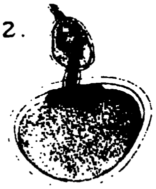

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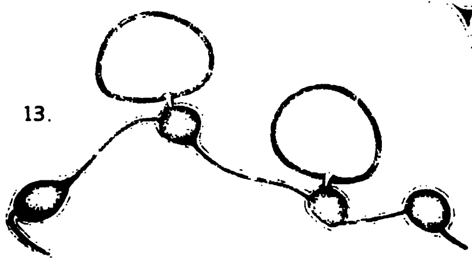

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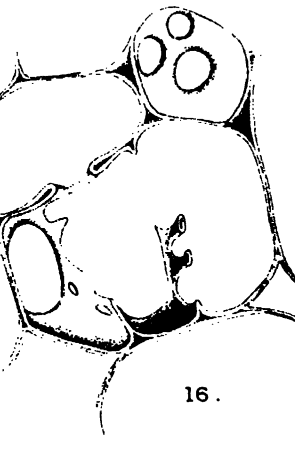

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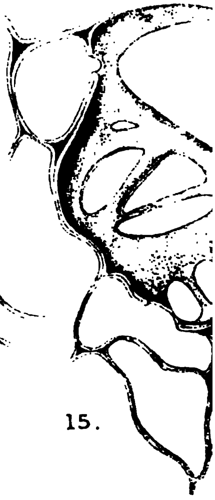

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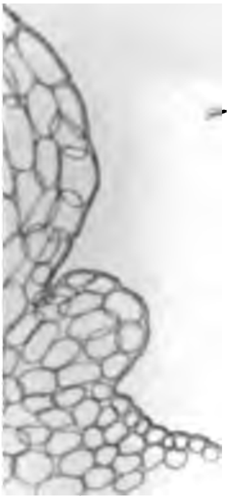
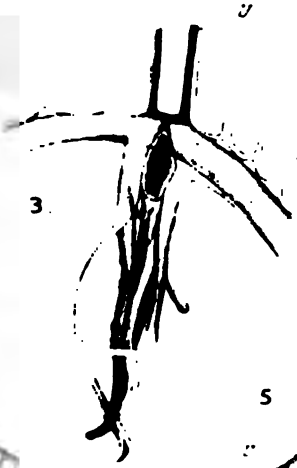
MAGNUS. — UROPHLYCTIS.





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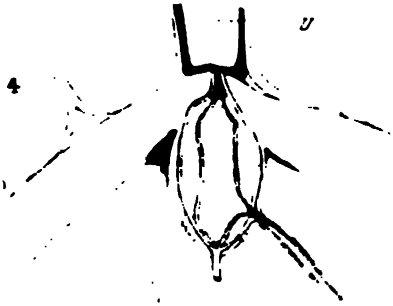

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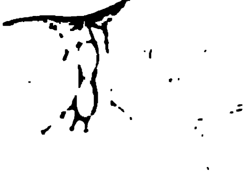

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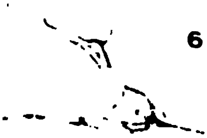

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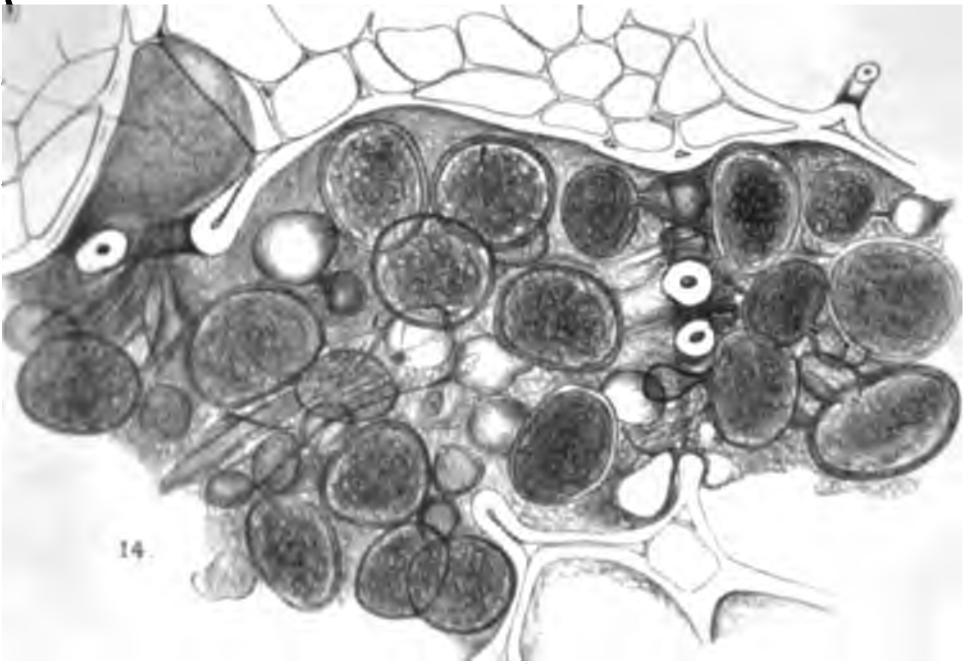

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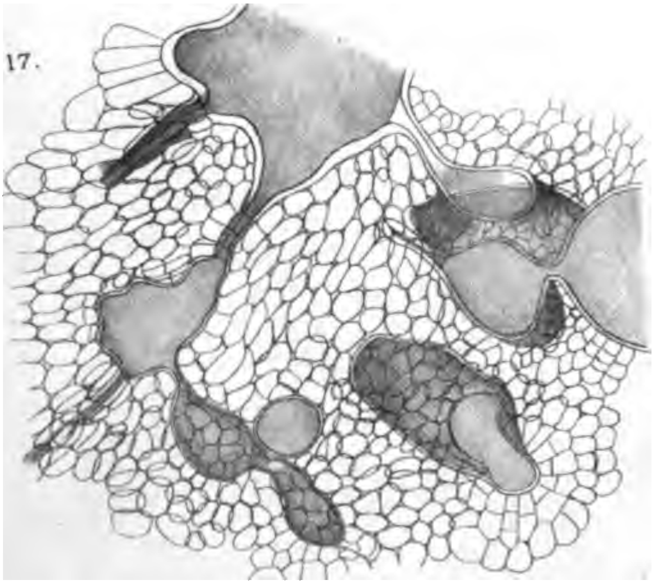

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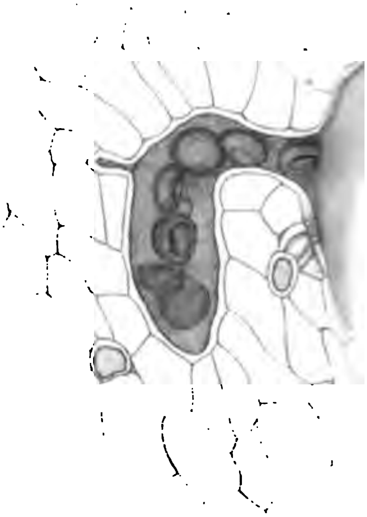

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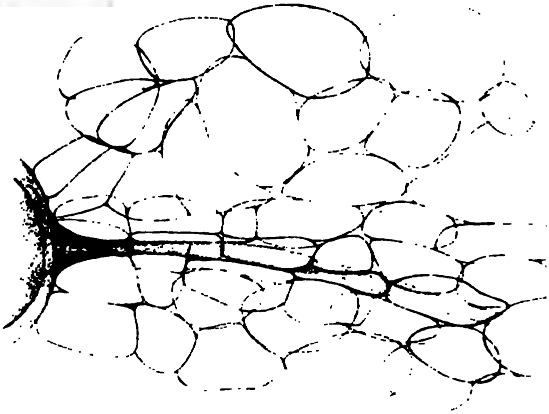

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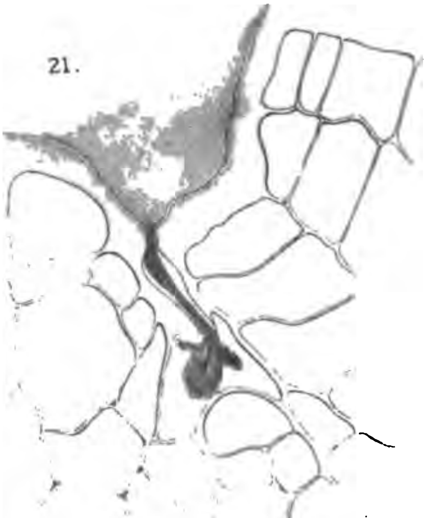

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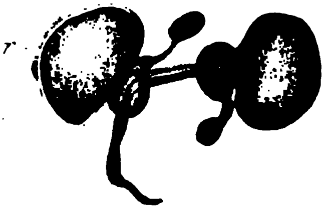

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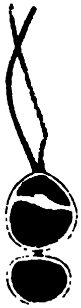

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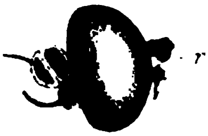

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MAGNUS.— UROPHLYCTIS.

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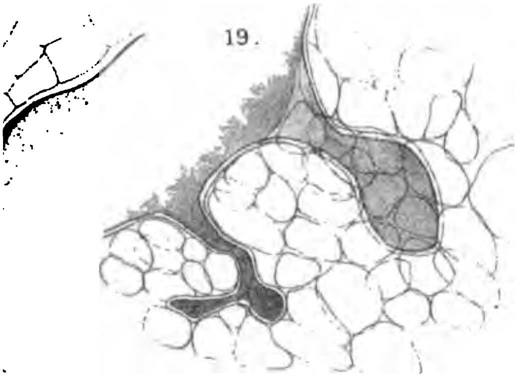

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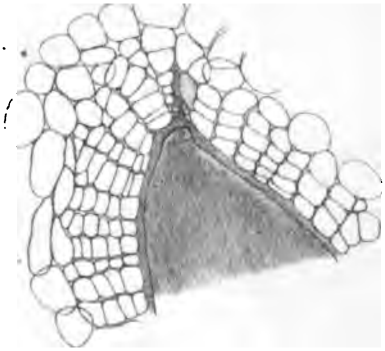

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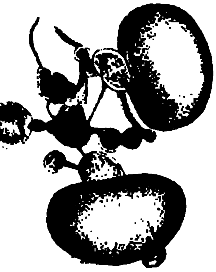

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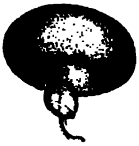

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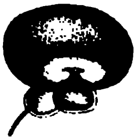

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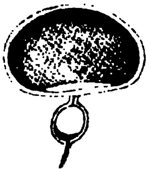

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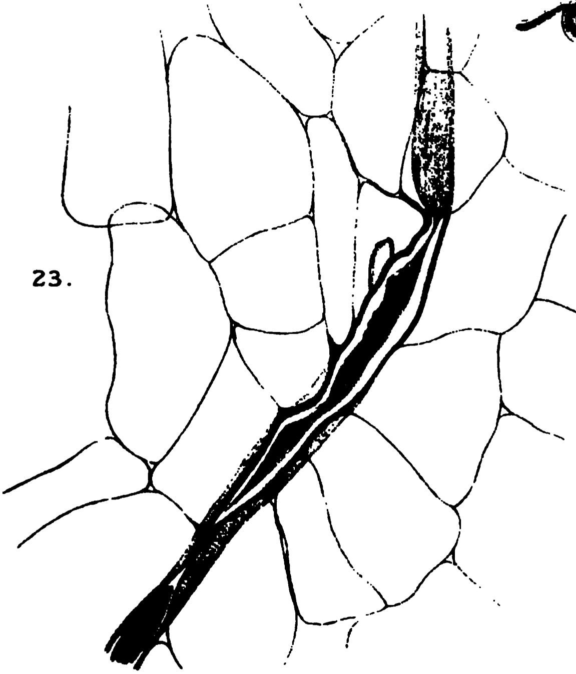

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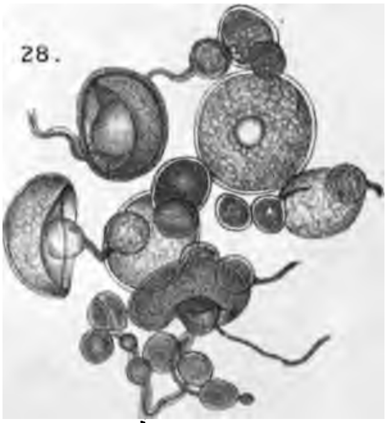

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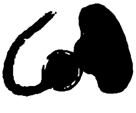

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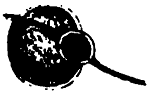

University Press, Oxford.

On the Polymorphism of the Green Algae and the Principles of their Evolution[1].

BY

DR. ROBERT CHODAT,

Professor of Botany in the University of Geneva.

THE classification of the Chlorophyceae has from time to time been the subject of numerous discussions, and even now it is by no means considered as settled. After comparing the different systems proposed by Rabenhorst[2], Gay[3], Klebs[4], De Toni[5] and others, it is easy to understand how it could be possible for so distinguished a botanist as Sachs[6] to say: 'It is certainly a great mistake to oppose the Chlorophyceae to the other Algae (Cyanophyceae, Rhodophyceae, Phaeophyceae) as a distinct type; such a division has nearly the same value as one in which the Phanerogams destitute of chlorophyll should be opposed to the green Phanerogams and in which both should be considered as distinct types. In the Chlorophyceae of the systematist several *archetypes* lie hidden each of which corresponds in its phylogenetic rank to the Archegoniatae, the Floridae, the Phaeophyceae, &c. It is not possible, however, in the present stage of investigation of the

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[1] [illegible] the British Association at the [illegible]

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Green Algae, to discover the archetypes in the chaos of the Chlorophyceae.'

Sachs[1] will for the present only recognize as archetypes the Cyanophyceae, Phaeophyceae, Rhodophyceae, Conjugatae (with Bacillariaceae), Siphoneae. and Archegoniatae. He includes the order Coleochaeteae in the Archegoniatae, separating it from the Green Algae—an arrangement which I am unable to accept, for reasons presently to be explained.

One cannot too strenuously oppose such a conception of the archetypes. It is very clearly evident that the most striking resemblance exists between the Phaeophyceae and the Chlorophyceae; the production of zoospores. the origin and development of sexuality, and the progressive development of the thallus occur in a similar manner in both groups. Their systematic rank as archetypes is of no higher value than that of the Bryophyta compared with the Pteridophyta.

Whilst agreeing with Sachs in regarding the Conjugatae as forming a very distinct order, I cannot go so far as he does when he removes them from the Chlorophyceae and groups them with the Bacillariaceae, a quite distinct and remote order belonging to the Phaeophyceae.

Just as we find in the Archegoniatae several distinct groups, such as the Mosses, Ferns, Equisetaceae and Lycopodineae, which cannot at present be traced back to any known common ancestor, so there are in the Chlorophyceae such orders as Oedogoniaceae, Sphaeropleaceae, Conjugatae, and Siphoneae, which are only separated from the others by certain tendencies already known in the whole group.

The Conjugatae, for example, are chiefly characterized by the mode of their fertilization. Conjugation, however, exists in certain species of *Chlamydomonas* (*C. Braunii*)[2]. Very highly differentiated chromatophores are also to be found in the Volvocineae[3], and the zygotes of these are not very different from those of the Desmidiaceae. Rooting hairs are not only known in the true Chlorophyceae but also in the Conjugatae[1], and the equality of the cells of the filaments is a very common character in both groups.

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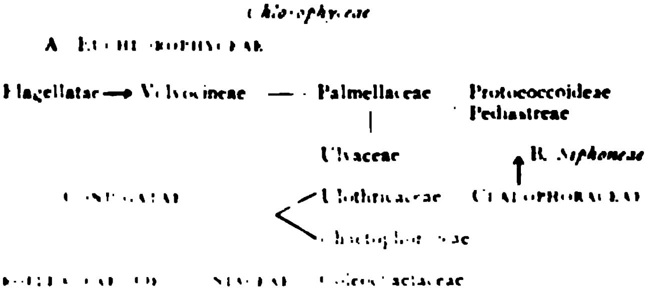
[1] Loc. cit., p. 201.

[2] Gorochankin, Beiträge zur Kenntniss der Morphologie und Systematik der Chlamydomonaden, Bull. Soc. Nat. Moscou, No. 3, 1890.

[3] See the above-quoted paper; also Schmidle, *Chlamydomonas Kleinii*, Flora,

With respect to the Siphoneae, which constitute a very special group, I think that the Vaucheriaceae can be detached from them with even more reason than the Conjugatae from the true Chlorophyceae. The multiplicity of the nuclei is not only common to the Siphoneae but also to many Chlorophyceae at an adult stage of their cells (Cladophoraceae, some Pediastreae)[2].

In this paper I shall set aside those orders which by the fixity of their characters, or their morphological differentiation, constitute very peculiar and definite groups among the Chlorophyceae.



As in similar cases, the proper course to adopt in order to discover affinities is to ascertain the complete development of each type under consideration, and to find out the modifications it undergoes under various circumstances, both in its natural habitat and also under cultivation.

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To know some, or even all, of the constant characters only is in no way sufficient; because if they are absolutely constant, the groups which are endowed with them become very sharply delimited, and their relation with other groups is very obscure, as for instance in the Characeae. It is only when we find representatives in which the important characters are altered or combined with peculiarities recalling other groups that we are able to put forward a theory as to the affinity of the family. The theory will be of no value until the induction drawn from comparative morphology leads us to find in the general life-history of the organism some gradual analogy showing a very definite tendency.

Thus, for instance, in the Archegoniatae the archegonium and antheridium (putting aside the details of their organization) are very constant characters; whereas we see in the reduction of the number of spores, or the progressive reduction of the prothallium, analogies tending to a very definite end.

In natural groups there are sometimes concomitant characters which may not perhaps be mutually dependent but which generally vary at the same time. Every peculiarity is, as we know, susceptible of more or less change, sometimes so much so that it becomes not apparent, but only potential. Thus, for instance, a plant apparently destitute of leaves may bear them when circumstances are more favourable; this is an indication that the want of leaves is only an adaptive character. In some cases the leaves are only found in the younger stages, the plant being afterwards entirely destitute of them. In this case the evolution or life-history of the plant in its totality will be necessary in order to determine its affinities.

For this reason I hoped to derive some fresh information from the variability, or, as it has been often called, the polymorphism, of the lower Chlorophyceae.

The theory[1] put forward in this paper is the result of a great many observations extending over several years, on material obtained partly in nature and partly by the aid of pure cultures, some of them under the microscope. I have drawn no conclusions from my work which are not based—firstly, on the fact that on the same plant (filament or colony) forms which had been formerly considered as distinct grow in direct connexion (*Cystococcus*, *Pleurococcus*); secondly, on the observation under the microscope of the production of spores and zoospores in plants which up to now were considered destitute of them (zoospores of *Eremosphaera*, spores of *Scenedesmus*, larval condition of *Chlamydomonas*, &c.); thirdly, on the fact that in a pure culture all the individuals, or nearly all, underwent a transformation which could be easily followed (filament of *Pleurococcus*, the fixed forms of *Raphidium*, &c.); and, fourthly, on the observation that under changed conditions the new forms are always in relation to their surroundings (*Palmella*, *Monostroma*, *Pediastrum*). All these observations have been verified a great many times[1].

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[1] See also Chodat et Huber, Remarques sur le système des Algues vertes inférieures, Archives Sc. Phys. et Nat. Genève, t. 31, p. 395.

Which are the lowest of the Green Algae it is not easy to say, because the most simple, namely, those from which I am inclined to derive the others, are still very highly organized, and those apparently less complex are merely reduced forms. Hence it would not be possible for a philosopher to take the lower Green Algae as a basis for the theory of the origin of life.

It is permissible, however, according to my observations, to trace the principal groups of the Chlorophyceae back to the little order of the Palmellaceae, in which are comprised the following genera: *Palmella* (*miniata*)[2], *Tetraspora*, *Gloeocystis* and *Apiocystis*.

Palmellaceae. Their gelatinous general envelope is produced by the confluence of the special gelatinous cell-walls. In *Palmella* the cells are grouped together in all directions of space, whilst in *Tetraspora* they are regularly disposed

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[1] [illegible] of polymorphism [illegible] Ha[illegible] and which were [illegible]

[2] [illegible] Bull. de [illegible]

in one plane. The cell divides in two distinct ways. In the first, after the first or second segmentation within the gelatinous cell-wall of the mother-cell, the cell-contents are arranged as in *Tetraspora*. This arrangement I have called *tetrasporoid*, or, when the dividing new cell-walls are more consistent, *pleurococcoid*. This has been very often described as *vegetative division*.

When the cell-wall is more roundish the new cells have a tendency to group themselves in three directions, viz. as if occupying the angles of a tetrahedron: this is the tetrahedric division, commonly manifested in *Gloeocystis*[1]. This I shall call *Gloeocystis-division*.

The cells of the Palmellaceae in every stage are, on leaving the envelope, capable of swarming as zoospores. As the cells vary greatly in size there is also great variety in the zoospores.

In certain conditions, as for instance when the solution in which the Algae are cultivated becomes more concentrated, the cell-walls grow more consistent; the products of division not being able to separate themselves from each other, division goes on, and then this cell is characterized as a sporangium. By the absorption of the separating cell-walls in the interior of the mother-cell, the daughter-cells can assume their rounded form.

Between this formation and the *Gloeocystis*-stage there is only a difference in the consistency of the wall of the mother-cell, and in the degree, more or less, of absorption of the separating walls of the daughter-cells. Very gradual intermediate conditions are also to be found, and are the best demonstration that in such lower forms no true distinction is to be made between the so-called free cell-formation and the vegetative division. This is very clearly shown in the genera *Monostroma*[2], *Palmella*, and especially in *Pleurococcus*.

From these three principal conditions, which are very equally represented in the true Palmellaceae, we can derive the three important tendencies which rule over the lower Green Algae.

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[1] See Gay, loc. cit., p. 92.

[2] See Chodat, Remarques sur le *Monostroma bullosum*, Bull. Soc. Bot. France, Session Extraordinaire, 1894, t. 41, p. cxxxiv.

A. The *zoospore-condition*, namely, the unicellular motile stage, becomes the leading principle in the Volvocineae, in which the two other conditions are only transient or subordinate.

B. The *sporangium condition*, that is to say the unicellular motionless stage with a consistent cell-wall, is in the second group the leading character, the two others being only accidentally realized, or merely transient (Pleurococcoideae).

C. The *Tetraspora-stage*, namely, where the non-motile cells are connected by regular cell-walls at right angles, or nearly so, arising from the division mentioned above, becomes a preponderant character in the Ulvaceae and the filamentous Algae by the increasing of the consistency of the walls, which is the condition of a more definite form of tissue or filaments; whilst the two others are merely transient or reduced.

These principal lines being established, each of them may follow a very logical evolution and conform to the leading principle. In their lower types there will be a less strong fixity of the main characters, and a much greater resemblance to the different conditions of the Palmellaceae.

Volvocineae. The general cell-structure of the Volvocineae is the same as in the Palmellaceae, but with variations due to their special evolution and to their mode of life. In some species of *Tetraspora*, and especially in the curious genus *Apiocystis*, each cell of the thallus is already provided with two cilia but these cilia are motionless.

The unicellular *Chlamydomonas* species, with their gelatinous envelopes, are propagated by bipartition or multipartition within the mother-cell wall; the division is at first longitudinal, but in most cases very soon becomes transverse by the rotation of the separating wall. Very often the four daughter-cells show a tendency to take the form of the

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[illegible]

mother-cell by the absorption of the dividing walls within the latter, which is then to be compared to a sporangium with 4–8 or more zoospores. These are set at liberty by the rupture of the sporangium-envelope.

In some cases, though rarely, the sporangial division passes into the *Gloeocystis*-stage or condition by the gelification of the wall of the mother- and daughter-cells (*Chl. pulvisculus*, *Chl. intermedia*).

A very interesting condition is that which I have described in the case of *Chl. intermedia*[1]. We have here a reduction to a *Tetraspora*-type, in which the cells are grouped in a single plane. By the further division of each of the four cells this stage is able to assume a very singular form, which I have named the *larval stage*. In this case each segmentation in the four cells is in a direction perpendicular to the division of the next cell. A similar mode of segmentation is well known in certain Volvocineae (*Volvox* and *Eudorina*)[2]. I have observed it in all the genera of this family, and also in the lowest forms, such as *Chlamydomonas*. This condition has been called the *Gonium*-stage. It is only a modification of the ordinary *Tetraspora*-division, for it may already be observed in the true Palmellaceae. The only difference is that here the cell-walls separating the products of the division are only subsequently gelified, so that all the cells lie very close to each other, forming a very peculiar plate-like body.

In *Chlamydomonas* variations are chiefly found in the cell-contents; the chromatophore may be continuous or perforated, with or without one or several pyrenoids; the latter are also variable in form in the different species. The species of *Sphaerella*, with their envelope remote from the body, are also closely related to *Chlamydomonas*; in the earlier stage[3] they cannot be distinguished from that genus, and the wall is close to the body. For this reason some young stages of

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[1] Bull. Herb. Boiss., 1894, loc. cit.

[2] Goebel, Ueber *Eudorina*, Grundzüge der Systematik, p. 41, Fig. 17.

[3] Cohn, Nova Acta Leopold XXII. Also, Chodat, Neige rouge du Col des Ecandies, Bull. Herb. Boiss., 1896.

Sphaerella have been described by several authors as new species of *Chlamydomonas*[1] (*Chl. nivalis*, Lagh., *Chl. sanguinea*). In some forms of *Chlamydomonas* the cell-walls become more and more reduced and are sometimes altogether wanting. From these forms to the metabolic species and genera[2], such as *Chloraster*, *Pyraminomonas*, and *Polyblepharis*, the passage is easy.

The genus *Gonium*, which has been separated in nearly all the systems from *Chlamydomonas* and placed in a different family, is very closely related to the latter. They may be considered as Volvocineae in which the tetrasporoid-stage is undergone in a motile condition. *Gonium sociale* can be very easily reduced to a unicellular form which can multiply in this stage for a certain time, as is shown by our pure culture experiments. A *Gloeocystis*-condition is also very easily obtained by gradual concentration of the nutritive solution. *G. pectorale*, which at its normal stage is a sixteen-celled motile colony, has been reduced in our experiments to four-celled colonies, which are very like *G. sociale*. We could only determine by further cultivation to which of the two species such forms belonged. In these young stages of *G. pectorale* each cell of the four-celled colony divides into sixteen, and thus produces successively or simultaneously four new sixteen-celled colonies. This can be very clearly verified in cases where only a single cell of the tetrad divides. This species is also capable of subsisting in a unicellular stage.

In *Pandorina* the earliest stage is the *larval condition*, which is transformed by degrees into a globular condition by the rolling in of the edges: in certain cultures of this species, however, I could obtain motile stages of the shape of *Gonium pectorale*[3], only less regular; and also by gelification, resting-stages like the *Gloeocystis*-stage of *Gonium sociale*.

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[1] Lagerheim, Die Schneeflora des Pichincha, Berichte der Deutsch. Bot. Ges. 1892, p. 517, tab. xxviii.

[2] See Dill, Die *Chlamydomonaden* und Verwandte, Jahrbucher fur wissensch. Bot. xxviii. 1895.

[3] Loc. cit., Materiaux pour servir, &c., I, pp. 593 and 597.

The origin of the thirty-two cells of the coenobium of *Eudorina* is the same as in *Pandorina*; we have here merely the larval stage more advanced in division, following the same law. By degrees the thirty-two cells thus formed group together into an ellipsoid, which at first is open at one end. Up to the adult condition this origin is still apparent, the two poles being different. Non-sexual reproduction by the formation of a coenobium of four to eight cells arranged as in *Pandorina*, viz. close to one another and not surrounding a gelatinous body, is very often produced in our cultivations. These may be easily distinguished from *Pandorina* by the mode of attachment of the cilia and the more globular shape of the cells.

The origin of a *Volvox*-colony is quite the same[1]. The larval-condition is here still more pronounced than in the others; but owing to the greater specialization of the individuals composing such a colony, the polymorphism is less marked. A unicellular stage is up to the present unknown. Thus considered, the Volvocineae constitute a very natural group in which the prevailing tendency is to replace the resting-stage of similar Palmellaceae by the motile condition. The evolution of the group can be followed step by step. As has been demonstrated in *Chlamydomonas*, we can trace two different lines of development; one has a tendency to retain the unicellular condition, the other to form a more or less complicated colony.

The possibility of an indefinite variation in *Chlamydomonas* is also very clearly shown by the variability of the cell-contents, which are very uniform in the other Volvocineae, and still more in the different forms of sexuality found in this genus. Isogamy, heterogamy, and conjugation[2] have been observed; whilst from the isogamous *Chlamydomonas* or isogamous *Gonium*[3] to the very highly differentiated antherozoid and oosphere of *Volvox*, there are the two graduated intermediate stages in *Pandorina* and *Eudorina*.

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[1] See Kirchner, Stein und Klein, in Pringsheim's Jahrb. f. Wiss. Bot. XX, and in Cohn's Beitr. zur Biolog. III.

[2] Gorochankin, loc. cit.

[3] Chodat, Materiaux, I, p. 597.

Protococcoideae.—In the same way that it is possible to derive the Volvocineae theoretically from the Palmellaceae by the prevalence of one of their peculiarities, namely, their motile stage, it is not less natural to imagine a similar origin for the Protococcoideae, in which group the motionless sporangium-stage is the most important.

As the close connexion between the two groups is not so clearly apparent as in the former case, we may consider the Protococcoideae either as the product of a special evolution from a reduced stage of filamentous Algae; or else, as appears to me more natural, as the direct product of the evolution of sporangium-conditions in supposed Palmellaceae.

In the lower forms there are globular cells with smooth or more or less ornamented cell-walls; the latter are generally the firmest. Their propagation takes place by the development of zoospores or spores. All their reproductive processes, however, show a very marked tendency to transform these motile elements into resting-spores.

We have in *Golenkinia radiata* (Chod.)[1] a particularly instructive case. The globular cell has a rather thick cell-wall with filiform radiating prolongations; multiplication takes place by discharging four naked and half-amoeboid spores, which soon become clothed; in other cases the cell can be revived by the extrusion of its cell-contents provided with a new smooth though thin cell-wall. Multiplication in this stage takes place by the production of quadriciliate zoospores. I have also described the same in the case of a gelatinous *Gloeocystis*-condition. The production of motile elements is replaced in the adult stage by naked non-motile spores, which under special conditions may be transformed into very definite spores with thick cell-walls.

Palmellococcus[2] is a genus producing in its natural habitat

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[1] Chodat, *Golenkinia radiata*, Journ. de Botanique, 1894, tab. 8, p. 305.

[2] Chodat, Matériaux, I, p. 600, loc. cit.

(wet walls) from two to four spores with rather thick walls. Simply by cultivation in common water the production of numerous naked spores is obtained in a few hours. They are, as in the former case, extruded by the gelification of the separating walls, and finally this substance disappears by solution. The nature and persistency of this material plays a very important part in the evolution of this group, as will shortly be shown. The limit of the number of normal spores is as a general rule two or four; the maturation of these spores is rapid, and when they are discharged from the mother-cell they have already acquired the peculiarities of the latter. In this case it is not surprising that the production of zoospores takes place so rarely.

The form of the mother-cell is in the lower species, or in the lower conditions, globular, but in some other species or adult conditions it becomes altered, and a great variety is found among the representatives of this group (*Oocystis*, *Kirchneriella*[1], *Lagerheimia*[2], *Nephrocytium*).

These different forms existing, two kinds of reproduction can take place. First, the cell-contents being divided into four (as is the case in most of them), these are sometimes only discharged very tardily. Growth takes place within the mother-cell, and by direct heredity these spores assume by degrees the same form as the mother-cell. The most striking example is given in *Lagerheimia genevensis*, a single-celled ellipsoid form, with four to eight long prolongations; the spores at the moment of their expulsion, and even before, are provided with the same appendages. Such a spore, having at the moment of its extrusion the form and external peculiarities of the mother-cell, has been named by me an *autospore*.

In such cases this mode of reproduction, although the commoner and more fixed mode, can be replaced under other conditions by true spores, and also, but very rarely, by zoospores, from which the others are derived, as is in some cases very clearly shown, partly by experiment, partly by comparison with allied species.

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[1] Chodat, Bull. Herb. Boiss., 1895, p. 301, Fig. 3.

[2] Sur le genre *Lagerheimia*, Nuova Notarisia, 1895, p. 86.

Sometimes the autospores are after their extrusion quite free (*Lagerheimia*, *Oocystis* p. p., *Dactylococcus*), sometimes they are surrounded by the gelatinous envelope formed as stated above; this is the case in *Kirchneriella*, *Nephrocytium*, &c. Lastly, the autospores which are not in this case surrounded with this jelly, are united together by threads formed of this substance, as in *Dactylococcus infusionum*[1]. It will be easily understood that under such conditions the arrangement is very variable, the cells being attached together either in one continuous row or in radiating threads, or forming a branched gelatinous filament.

In this case the form of the mother-cell is susceptible of great variation (in the same species), the more modified form being a spindle-shaped cell. All these forms may be obtained in a pure culture at the same time by gradual transformation. When the four spindle-shaped autospores are not set free by the solution of the gelatinous matter, they often remain attached in a single row as they were in the mother-cell, and by their expansion in a plane constitute the well-known coenobium of *Scenedesmus acutus*. All these transformations, and those above described, I have observed a great many times, and it is very easy to repeat them.

As a further step, this little coenobium producing similar autospores united together in a row at the instant of their expulsion, the production of an *auto-colony* takes place. Under commonly observed conditions this so often occurs that it has been considered as the only mode of reproduction in *Scenedesmus*. It becomes more constant, however, in some other species of the same genus, such as *S. quadricauda*, which is generally provided with long spines. In *Raphidium*[2] we have in a way a repetition of this method of propagation; the long spindle-shaped cell divides within the thin mother-cell twice transversely; the four superposed cells show a tendency to elongate and to assume the spindle form of the mother-cell. Finally, by absorption of the cell-wall they are set free, as though they had originated by simple fission.

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[1] Chodat et Malinesco, Sur le polymorphisme du *Scenedesmus acutus*, Bull. Herb. Boiss., 1893, t. 1, p. 184, et ibid., 1893, p. 640, et Chodat, Matériaux, I, pp. 602, 604.

[2] Chodat, Matériaux, I, p. 608, tab. 26.

When there are some filaments of *Vaucheria* or other Algae in a culture of *Raphidium Braunii*, the latter plants develop a little disk at one end by which they become attached to the filament. In this case the difference between the two poles is clearly demonstrated by the behaviour of the products of division. In most of the individuals so attached, the wall of the mother-cell becomes more resistent at the base, and only the upper part is dissolved; the new *Raphidia* are discharged and very often remain attached to the edge of the cupule-like mother-cell. This process being repeated, a very elegant bush-like colony arises, and a certain similarity to the case of *Sciadium* is produced.

The polymorphism of this species is very considerable. A similar evolution of the fixed stage of *Dictyosphaerium* has also been described after repeated observations.

All these different Protococcoideae can be reduced to unicellular globular conditions, in which they behave like the sporangia of *Palmella* or *Dactylococcus*[1]. I have also obtained from all the genera a gelatinous condition like *Palmella* by the suppression of the motile element and the disappearance of sexual differentiation. Up to the present time isogamy has been described in only a very small number of species.

The **Pediastreae** constitute only a parallel group or suborder to the former one.

As I have just shown that the coenobium of *Scenedesmus*, *Raphidium* and others can be derived from unicellular and spore-producing types, the same theory is also applicable to the Coenobieae. Leaving aside *Hydrodictyon*, which seems to be a very peculiar type, the others are grouped in several small series.

The first series has as its higher type the curious genus

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[1] Chodat, Bull. Herb. Boiss., 1895.

Hariotina, and as intermediate forms the different species of *Coelastrum*.

It may be imagined that *Coelastrum sphaericum*, for example, has been derived from a lower form of the Protococcoideae with autospores. As in the former series, the spores may be at the time of their expulsion either free or united together by intermediate mucilaginous matter. In such a theoretical type the autospores would have been grouped in a globular colony. This may often be observed in some very doubtful species, such as the so-called *Chlorococcum infusionum*, *Pleurococcus angulosus*, &c., described by Artari[1].

One of the causes of the production of auto-colonies, in this and in other cases, is the rapid multiplication of the nuclei. At a very early stage of their development after their extrusion, the cells of the Pediastreae contain several nuclei (4, 32, 64). By direct heredity each of the cells assumes within the mother-cell the shape of the parent, and when the mother cell is provided with sculptures or prolongations, as is the case in some species of *Coelastrum* and in *Hariotina*, these appendages are already formed at the instant of the extrusion of the new colony.

This theory of the evolution of the group may be demonstrated by the very specific variations of the different species of *Coelastrum*, for example *C. sphaericum*[2]. In the ordinary condition the cells of the coenobium of this species remain united together at the time of the birth of the young colonies. The cells of the parent colony are for the most part emptied of their contents; in other cases each of the cells of the coenobium is separated, and can produce a new colony in this condition of isolation, or else may give birth to a two-celled body, as occurs in *Sorastrum*, in the cells of which new colonies are formed. Lastly [illegible] or multi- [illegible]

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[1] [illegible]

[2] [illegible]

In a similar manner *Sorastrum* [1] may be derived from some unicellular *Polyedrium*, as I have shown; and the same thing is highly probable with respect to *Pediastrum*, which is connected with the unicellular *Polyedrium*-forms through the interesting genus *Euastropsis* [2]. But in this latter small group curiously enough the zoospores are still present; discharged as in other cases by the walls becoming gelatinous, they remain enclosed in the vesicle, and their evolution takes place within it. Finally, a new coenobium is formed. I have demonstrated by experiment that it is possible to induce *Pediastrum* to form the new coenobium directly within the mother-cell without the intermediate stage of the enclosed motile cells. By concentrating the nutritive solution, *Pediastrum* produces new colonies in the manner shown in *Coelastrum*, i. e. typical *auto-colonies*.

In *Hydrodictyon*, which seems to be a very special type, an intermediate condition between *Pediastrum* and *Coelastrum* occurs. The swarming stage is attained not, as in *Pediastrum*, outside the mother-cell, but within it. The question whether *Hydrodictyon* properly belongs to this group seems to me an open one. Perhaps the likeness to the Pediastreae is due to a convergence rather than to a common origin. There are in fact so many analogies between *Cladophora* and this genus in the mode of formation of the zoospores, and the constitution of the cell, that a new line of comparison is required.

To sum up: we have in the Pediastreae a repetition of the evolution of the other Protococcoideae, and a great analogy with the Volvocineae in the complication of the thallus, though for reasons which are quite independent in both groups.

Ulvaceae-Chaetophoraceae. The type which is realized in the Ulvaceae is still essentially similar to that of the true Palmellaceae. In fact there is only the greater consistency of the walls and, consequently, the more definite form of the thallus. It is true that here the cells of the thallus are more regularly disposed, and the number of the cell-rows also is determinate. With the gradual evolution in the external morphology, the thallus of the marine species becomes very definite, and in some cases, like *Enteromorpha*, active apical cells play a predominant part in the development of the new tissues. Through the genus *Monostroma*[1], and especially through the fresh-water species, the Ulvaceae are closely connected with *Tetraspora*. The disposition of the cells dividing at right angles, above described in the Volvocineae and Palmellaceae, is here also of very common occurrence. With regard to the early development, there are several very variable modes. The young thallus may take a globular, tetrasporoid, or shortly filamentous shape. The Palmella-condition is also found under certain circumstances in *Monostroma*, and resting cells with thick cell-walls are very easily formed, as in the Palmellaceae. The production of macro- and micro-gametes or zoospores takes place in the same manner, and isogamy is quite as frequently observed. There can be no doubt, therefore, about the close affinity of the Ulvaceae to the Palmellaceae.

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[1] See De La Rue, Sur le développement du *Sorastrum*, Ann. Sc. Nat., sér. V. t. 17, 1873, and Chodat, Évolution des *Coelastrum*, loc. cit., p. 275.

[2] See Lagerheim, Ueber Arktische Kryptogamen, I. Tromsö-Museum, 1894.

The same may be said of the Chaetophoraceae, which constitute another group. The polymorphism of *Stigeoclonium* is well known[3], this Alga with its branched filaments can assume a *Palmella*-condition[4], and in every stage of its life exhibits some very striking modifications. The adult plant consists of a rhizome with branched filaments and swollen cells, from which rhizome arise further branched filaments terminating in hairs. But the ramifications are very inconstant, and consequently the different species have not been distinguished with accuracy. Each of the cells can separate[5] from the whole by forming a macro-zoospore (as in Palmellaceae) or by transformation into a *Palmella*-stage. The first modification takes place generally in the erect filaments, the second is more commonly found when the plant is in the rhizome stage. Each of these cells can form a globular sporangium with 4–8 quadriciliate zoospores; in other cases subdivisions are effected, as in *Tetraspora*, and these can gradually pass into a *Gloeocystis*-condition or *Protococcus*-condition for the same reasons as above indicated in the Palmellaceae. In the *Protococcus*-stage (sporangium-condition) the zoospores can be transformed into spores which by mutual pressure become more or less polyhedric. After the destruction of the cell-wall these daughter-cells may remain united together and constitute multicellular bodies, from which zoospores or spores similar to those of the mother-cell escape.

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[1] Chodat, *Monostroma bullosum*, Soc. Bot. France, loc. cit.

[2] Reinke, Ueber *Monostroma bullosum*, &c., Jahrb. f. Wissensch. Bd. II, p. [illegible] 1878.

[3] Famintzin, Die anorganischen Salze [illegible] Bull. de l'Acad. Imp. des Sc. St. Pétersb., XVII, p. [illegible]

[4] Cienkowski, Zur Morphologie der Ulothricheen, Bull. de l'Acad. Imp. des Sc. St. Pétersb., XXI, 1876.

[5] Chodat et Huber, Remarques sur le système des Algues Vertes, Archives des Sc. Phys. et Nat., Genève, 1894, p. [illegible]

But I have also observed the same thing in the erect filaments and also in the gelatinous condition. In all the lower Chaetophoraceae, *Ctenocladus*, *Chaetonema*[1], *Endoclonium*, similar conditions also exist. In the genera *Chaetophora* and *Draparnaldia* this is less marked and confined to special developments.

This polymorphism, which shows us so clearly the relation of this group to the Palmellaceae, is of the greatest interest, and is similar to, though not identical with, the following, studied by me in *Pleurococcus vulgaris*.

This plant is apparently well known. It grows on the bark of trees, on wet stones, on turf in mountainous places or on bare wood in damp places, and is of a characteristic green colour. In opposition to Gay, Artari, and Klebs, who believe in the fixity of the form of this Alga, I have arrived at the conclusion, after three years of continuous research, that its variability under different circumstances is very great. Under this name we must include not only the common form without a pyrenoid, but also the similar one with a pyrenoid (*Pl.*

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[1] Huber, Sur un état particulier de *Chaetonema*. Bull. Herb. Boiss., 1894, p. 164.

simplex, Artari[1]), as well as such very different forms as *Cystococcus* and *Protococcus vulgaris*.

This plant having been considered as the type of the so-named 'Pleurococcaceae' by recent authors, such as Klebs, Wille, &c., it is necessary to give somewhat full details concerning it.

As generally described in its natural and commonest condition, the four cells are arranged in one plane, and each cell is characterized by a parietal chromatophore. By repeated divisions very regular thalli are formed, in which the cells lie close together. This very common condition of *Pleurococcus vulgaris* is the aerophilous form, which produces a more or less thick stratum on the bark of trees. In a nutritive solution the culture of this plant, gathered in very dry places, is often impossible. When, however, in such a solution, the aeration is good, some, or all, of the individuals grow very well, and after some days or weeks the formation of a short filament begins by the protrusion of a prolongation continuous with the *Pleurococcus*-cell. In some cases, out of the four or eight cells of a thallus, only a single one produces such an expansion, so that it is unquestionable that the filament belongs to a true *Pleurococcus vulgaris*. In other instances from two to eight do the same. The growth of the filaments is always slow, and when they become more elongated division by segmentation occurs. This may also be observed in larger thalli which are covered with short or long hairs arising from nearly all the outer cells. When circumstances are favourable almost all the individuals develop in the same way, and a great many little branched or unbranched filaments spread in every direction. I have not been able to obtain filaments exceeding twelve cells in length; the thalli often appear to be more branched, but this is due merely to the fact that the four initial cells of the normal *Pleurococcus* from which the filaments originated still remain united; in this case the ramification is radiating. I have repeated these cultures a great number of times and have always obtained the same result.

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[1] [illegible]

It is not necessary, however, to have recourse to cultures in nutritive solutions; in wet years all these developments can be observed on the bark of shady trees exposed to rain and moisture. I have renewed these experiments by starting with *Pleurococcus* gathered in various localities in Switzerland and France, and have always arrived at the same result.

A second variation, which is quite as frequent, is the production of spores. In cultures or in fresh conditions this takes place sometimes by the gradual solution of the separating cell-walls of the thallus. During this process the cell-contents acquire a special envelope and become more and more globular. The general envelope of the thallus is not altered, but assumes the function of a sporangium-wall. In this case it may be that on a large thallus one half of the cells undergo this transformation, whilst the remainder retain their normal structure. Some of the cells of these small typical thalli may grow larger and become more globular; at length the latter are separated from the normal cells and constitute very different bodies, in which division of the contents generally takes place very soon, and spores are formed in this way. This production of sporangia is of frequent occurrence in connexion with the normal *Pleurococcus* cluster of cells, in which I have often observed not only the above-described sporangia but also at the same time more or less developed filaments. This shows very obviously how difficult it is, with regard to this and other productions, to discover the true cause of their formation, when they grow not only under the same conditions but even on the same plant.

Finally, each cell of the more or less complicated cluster of *Pleurococcus* can be renovated by a similar process as I have just described above for the formation of spores from the vegetative thallus; but in this case the renewed cells retain the same elongated or more or less quadrangular form, and are consequently very like some *Stichococcus*. But this is only an external resemblance. *Stichococcus* is quite a different plant, belonging to the Ulothricaceae. All these sporangium-formations I have obtained in my pure cultures, and have also observed them in their natural habitats on many occasions.

Naegeli has distinguished under the name of *Cystococcus* a unicellular Alga which he believed quite different from *Pleurococcus*. In his drawings the cells are globular, with a stellate chromatophore, and a very distinct pyrenoid, which has often been taken for the nucleus. On one side there is a clear space containing a nucleus. He also describes a cell-division in which the numerous daughter-cells are polyhedric by mutual pressure. It is well known that *Cystococcus* can produce biciliate zoospores. I have also observed very minute gametes fusing together a short time after their expulsion. This plant, if only known in this stage, could be taken as a type of the Protococcoideae, for in it may be very easily observed the transformation of the zoospores into non-motile spores. But *Cystococcus* is not a member of the Protococcoideae at all: it is merely a stage of the development of *Pleurococcus*.

I have already stated how in the same *Pleurococcus* the different cells can produce a globular sporangium with spores, a transformation of a part of the thallus into a false sporangium, and branched or unbranched filaments, and the same phases may be observed in the *Cystococcus* in its typical form, and the true *Pleurococcus*, which is provided with very different chromatophores. For instance, I have repeatedly noticed on the same thallus, with or without pyrenoids or filaments, typical *Pleurococcus* cells with parietal chromatophores, and one or more *Cystococcus*-cells with a more or less stellate chromatophore. In such cases I have seen the expulsion of the zoospores or gametes, whilst in other cells of the same thallus spores were formed[1].

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[1] [illegible] under the name of [illegible] chromatophores and [illegible] pyrenoids and with [illegible] I could not obtain motile elements [illegible] In all cultures the two remained quite [illegible].

The appearance of the chromatophore when the *Cystococcus* form is developed, or in a free condition, is, as usually described, somewhat stellate and always with an incision on one side. By division this appearance often changes into that of a flat cup, the upturned edges of which cause the cells to look as if there were parietal chromatophores.

I cannot sufficiently insist upon the fact that all these various formations are not only found separate and combined on the same spot of bark, but even on the same thallus.

Besides the formations here mentioned, the student can follow step by step in older and more concentrated cultures the development of very curious gelatinous conditions which are very similar to the genus *Hormotila* described by Borzi.

To sum up: *Pleurococcus*, in the various conditions of its evolution, may develop branched filaments, sporangia with zoospores, gametes, and spores. This is sufficient ground for removing this plant from the order Protococcoideae, and considering it as a type of the Chaetophoraceae, reduced owing to its existence as a lichen-gonidium.

In this order isogamy only has been described. In a recent paper one of my assistants, Dr. J. Huber[1], has carefully studied the curious heterogamy of *Aphanochaete*, of which I had at the critical moment collected specimens growing on *Oedogonium*. In this epiphyte the central cells of the bilateral thallus become greatly developed and produce oogonia. At this time the latter, like the other cells, are provided with a typical hair. The cells of the periphery contain the antheridia, which are much smaller and paler, sometimes colourless, and grouped in a very similar manner to the antheridia of *Coleochaete*. The oosphere, which is five to six times larger, is set free by the swelling of the inner side of the cell-wall and the rupture of the upper part of the oogonium. The expulsion takes place suddenly, and the large quadriciliate gametes remain enclosed in the mucilaginous hyaline vesicle, as is the case with the zoospores of the same plant, which likewise bear four cilia and are smaller. The male gametes are formed in the same way, rarely two in one cell, but the gelified vesicle is rapidly dissolved and fecundation can be effected. The fusion of the male gamete with the very sluggish oosphere, which is at this moment always in front of the empty oogonium, has been observed. It may be compared with the corresponding process in *Coleochaete*; in the two genera the oogonium and zoospores are homologous. The oosphere and antherozoid have the same shape and the same number of cilia as the zoospores. In *Coleochaete pulvinata*[1], for example, the oogonia and the cells which give birth to the zoospores are terminal cells. The antheridia in both genera are also of the same morphological rank; each antherozoid can be compared to a micro-zoospore. To this it may be added that the cells of *Coleochaete* are provided with chromatophores of the same shape, containing the same pyrenoids; and the hairs and zoospores are so clearly allied to those of the Chaetophoraceae and other Green Algae that we may justly feel greatly surprised to read in a serious paper that the Coleochaeteae[2] ought to be united with the Archegoniatae, and separated from the Chlorophyceae. It is true the author admits that there is no direct passage from the Coleochaetae to the Mosses and Ferns. On the other hand, this direct relationship does actually exist between this group and the other Chaetophoraceae. *Aphanochaete* is beyond all doubt the connecting link which unites the two nearly allied groups; in fact, the only difference between the two consists in the fact that in *Coleochaete* the oosphere is not motile, and remains enclosed in a sort of archegonium. But it must be remembered that in the other genus the oosphere is almost motionless and remains in front of the oogonium.

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[1] Huber, Sur l'*Aphanochaete repens*, Soc. Bot. de France, Sess. extraordinaire en Suisse, 1894.

If, as we are permitted to believe on comparison with similar cases, the oosphere is merely a macro-gamete which remains enclosed in the cell-wall, the stage of *Aphanochaete* is of the greatest interest, being the intermediate step between two motile hetero-gametes and the true sexuality realized in the higher types. The form of the oogonium of *Coleochaete* is simply a fortuitous resemblance to the archegonium, and is not sufficient to establish any homology.

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[1] Pringsheim, Jahrb. für wiss. Bot., Band II.

[2] Sachs, loc. cit., pp. 184, 185, &c.

The analogy with *Oedogonium*, in which the two gametes may be compared to two differentiated zoospores, is much more marked. The number of antherozoids arising from one cell is also comparable, and their origin is quite different from that of the antherozoids of Mosses and Ferns.

All this very clearly indicates a direct affinity between the Coleochaeteae and the Green Algae, which considered as a whole group (the Siphoneae put aside) is quite as natural a one as the Archegoniatae.

With regard to the so-called sporogonium of *Coleochaete*, there is no dispute about the statement that the ovum undergoes division after fertilization; but this segmentation, which reminds one to a certain degree of the production of the sporogonium in some lower Liverworts, shows a far greater resemblance to similar products in the Green Algae.

The fertilized zygote of *Hydrodictyon* divides during germination into several zoospores; the same thing is known to occur in *Oedogonium*. In some Volvocineae, such as *Gonium*, according to my observations, the zygote gives birth to four zoospores with gelatinous envelopes, whilst in others the zoospores are extruded before division.

From the fact that in the case of *Coleochaete* the ovum is more protected by the formation of a resistent envelope, germination becomes slower; and as I have already shown that the transformation of zoospores into spores and finally into autospores may be effected, it is evident that a similar adaptation takes place here.

The little thallus of *Coleochaete* cannot be contrasted with the antecedent plant as an entirely new formation; according to all analogies with other Algae, it constitutes simply the beginning of a normal plant, only modified under peculiar circumstances. Similar small thalli are moreover formed in *Aphanochaete*, but from zoospores. This is merely the result of normal activity due to fertilization, or the increase of nourishment.

Furthermore, we must admit that in the Green Algae antithetic alternating generations cannot be distinguished; for the production of zoospores, spores, and gametes depends on certain circumstances, and occurs in most cases at every stage of the life-history.

In conclusion I may say that all the facts above mentioned relating to the variability of the Green Algae help us in studying the difficult problem of the phylogeny of these plants. It seems to me that it is now easy to understand what are the leading principles in the evolution of the Volvocineae, Protococcoideae, and Ulvaceae. On the other hand, we can now go back step by step from the higher branch of the filamentous Algae, the Coleochaeteae, to the lower Chaetophoraceae, of which *Pleurococcus* is a genus in a stage of degradation. I could have shown in what manner the Ulothrichaceae are related to the Ulvaceae, by such a series of steps as *Ulothrix*, *Hormidium*, *Schizogonium*, and *Prasiola*. The Chaetopeltideae are likewise very closely related to the Ulvaceae, and especially to *Monostroma*. I hope shortly to complete in another paper this theory of the evolution of the Green Algae.

The true affinities of the Green Algae are not by any means so obscure as has been recently asserted by some of the most competent botanists; on the contrary, their phylogeny is especially clear; and the present paper may throw some light on this difficult subject.

Contributions towards a Knowledge of the Anatomy of the Genus Selaginella, Spr.

BY

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With Plate IX.

PART III. THE LEAF.

AMONGST the older monographs, the first important contribution to our knowledge of the anatomy of the leaf of *Selaginella* occurs in Hofmeister's treatise on the Structure and Development of the Higher Cryptogams[1]. Dealing more especially with the species *S. denticulata*, *S. Galeottei*, and *S. Martensii*, he first of all gives an account of the mode of development of the leaf, describing it as arising as a horizontal ridge of cells, embracing about one quarter of the circumference of the stem, and first appearing about eight or ten cells behind the apical cell. The cells forming the ridge undergo division in such a manner as to give origin to a plate two cell-layers in thickness, the central cells of the ridge dividing more actively than those nearer the edge. On either side of the middle line the cells undergo

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[1] Vergleichende Untersuchungen der höheren Kryptogamen. Leipzig, 1851.

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divisions parallel with the surface, forming a central core, which afterwards becomes the vascular bundle. At the same time the apex of the young leaf becomes elongated, and the cell-walls in this region become thickened. Division and growth now progress rapidly at the leaf-base, the cells of the lower surface being more active in this respect than those of the upper side. Hofmeister then describes the further modifications in the course of transition to the adult form, attention being drawn to the formation of marginal papillae and hairs, and the development of rows of warts on the cells near the margin of the leaf. He further points out that the median cells of the leaf are arranged so as to form a network with conspicuous intercellular spaces, whilst at the margins the mesophyll is wanting, and the upper and under epidermal layers are in contact.

Russow[1], in describing the leaves in the genus, speaks of the leaf as traversed by a single vascular bundle, which terminates before the apex of the leaf is reached. The xylem consists of spiral and reticulate elements, and is surrounded by a scanty phloem. No endodermis is developed. The ground-tissue, he states, consists of spongy mesophyll, well developed in the more robust species, but only surrounding the vascular bundles in those with more delicate leaves. Russow draws special attention to the epidermis of the leaf, and shows that it varies considerably both in the form and in the nature of the cells of which it is composed. In some species (e. g. *S. Kraussiana* and *S. Galeottei*) the upper and under epidermal layers are alike, and he suggests that probably the leaves of all species belonging to the section ‘ Articulatae ’ have this structure. Of Spring’s section ‘Articulatae’ (which is not synonymous with that of Baker) I have examined only one other species in addition to those mentioned by Russow, viz. *S. sulcata*, and in that species the upper epidermis is quite similar to the lower. In other cases, Russow continues, the cells of the ligular face are shorter than those of the aligular face, as in *S. Lyallii*, *S. cuspidata* and *pilifera*. In the majority of the species the epidermis of the ligular face consists of polygonal cells almost or quite isodiametric, and appearing conical in transverse sections of the leaf, whilst the epidermis of the aligular face consists of elongated cells rounded or quadrate in section. Russow further points out that with regard to the smaller (dorsal) leaves, the cells of the ligular face correspond to those of the aligular face of the larger (ventral) leaves, and those of the aligular face of the smaller leaves to the cells of the ligular face of the larger leaves. The elongated epidermal cells contain several small chloroplastids, the conical cells generally two, or occasionally three, chromatophores only. In some species (e. g. *S. Kraussiana*) a layer of conical cells occurs beneath the epidermis of the ligular surface of the larger leaves and of the aligular surface of the smaller leaves, whilst in *S. Lyallii* this rudimentary palisade-layer becomes well marked. Stomata are described as occurring only on the aligular face, but exceptionally in *S. pubescens* (= *S. Braunii*, Bak.) stomata occur on both surfaces of the smaller leaves [1]. Of the species mentioned by Russow as having stomata on the aligular surface only, I have examined all, and have been somewhat surprised to find that no less than three of them, viz. *S. Kraussiana*, *S. cuspidata*, and *S. pilifera*, had stomata on both sides of both dorsal and ventral leaves. Lastly, Russow calls attention to the peculiar cells of the epidermis of the ligular face of the larger and the aligular face of the smaller leaves (Doppelkegeln) in *S. uncinata*, and to the development of sclerotic fibres at the margins and on the surface of certain species e. g. *S. Martensii* and *S. stenophylla*.

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[1] Vergleichende Untersuchungen über Leitbündel-Kryptogamen. Mém. Acad. Imp St. Pétersb., 1872.

Treub [2] has studied the mode of development of the leaf in *S. Martensii*. His results agree in the main with those of

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[1] 'Bei *S. pubescens* finden sich auf beiden Flächen der kleinen Blätter Stomata; bei allen übrigen untersuchten Arten war nur die Epidermis der Aligularfläche, sowohl der grossen als kleinen Blätter, mit Spaltöffnungen versehen.' Russow, l. c., p. [illegible].

[2] Recherches sur la Végétation du *Selaginella Martensii*, Spr. Leide, 1877.

Hofmeister. I do not purpose in the present paper to enter into the question of the development of the leaf, reserving that point for discussion in connexion with the structure of the stem-apex on which I am at present engaged.

In a brief note read before the British Association in 1887, McNab[1] points out that in *S. densa*, Hort., *S. Poulteri*, Hort., and *S. Kraussiana*, A.Br., a triple row of stomata is developed along each margin, one row above, one below, and one actually on the margin itself. All three species want the sclerotic marginal cells so commonly found in that situation.

Haberlandt's important paper on the chloroplastids[2] may next be noted. In this treatise he gives a very full account of the structure and development of the chlorophyll-bodies. In the present contribution I have omitted any discussion of these, simply because I have nothing of any importance to add to Haberlandt's account.

I may next refer to Dangeard's account of the structure of the leaf[3]. He distinguishes two distinct types of leaf-structure: (*a*) where the upper and lower epidermal layers are similar, and (*b*) where they are dissimilar. Under the first type two sub-sections are constituted: (1) leaves in which the mesophyll is homogeneous, e.g. *S. spinosa*, &c.; and (2) leaves in which the mesophyll is heterogeneous, e.g. *S. Kraussiana*, &c. The leaves in the former sub-section have an epidermis composed of elongated (in the long axis of the leaf) rectangular cells, while the mesophyll consists of long, branched, narrow cells with numerous intercellular spaces; in the latter section the mesophyll is subdivided into two distinct layers, an upper palisade-layer composed of conical cells perpendicular to the epidermis, and a lower spongy layer. The type of leaf with dissimilar epidermal layers Dangeard further describes as having homogeneous reticulate mesophyll throughout.

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[1] On the Stomata and Ligules of *Selaginella*. Brit. Assoc. Reports, 1887.

[2] Die Chlorophyllkörper der Selaginellen: Flora, 1888.

[3] Essai sur l'Anatomie des Cryptogames vasculaires: Le Botaniste, 1889, p. 247.

The stomata he describes as distributed, as a rule, over the midrib on the aligular face of the leaf, more rarely over the entire aligular surface. In the Rosulatae group (e. g. *S. convoluta*, *S. cuspidata*, &c.) stomata, according to Dangeard, occur on both sides of the leaf. He also draws attention to the sclerotic cells of the epidermis of several species, and to the uni- (or more rarely pluri-) cellular hairs which occur along the margins of some leaves. Dangeard finally describes the vein as composed of a small cluster of protoxylem-elements only surrounded by phloem, adding 'le faisceau de cette nervure ne possède jamais de métaxylème comme celui de la tige.' On this point he is at variance with Russow, who speaks of the xylem as being composed of 'Schrauben- und Netzzellen.' In *S. sulcata* Dangeard describes the phloem as limited to the under surface of the bundle, and points out the occurrence of bast-fibres (fibres libériennes) in the phloem of *S. stenophylla*. He again differs from Russow in asserting the presence of an endodermis, though 'certainement mal différencié.' Wojinowić [1], in his thesis on *Selaginella lepidophylla*, gives an exhaustive account of the structure of the leaf, calling attention especially to the presence of stomata on both sides of the ventral leaf, and to their absence from the aligular face of the dorsal leaf. He says, speaking of the latter, 'Die Epidermis dieser Seite (der organischen unteren, vom Stengel abgewendeten Seite) führt keine Spaltöffnungen,' and again 'In der Epidermis der organischen Oberseite (d. h. der dem Stengel anliegenden) finden sich Spaltöffnungen.' In this statement he is supported by Erikson (*l. c.*), who says, in discussing the dorsal leaf, 'Epidermis på den undre (uppåtvända) ytan bestar af langstrackta celler med tunna, nastan raka mellanväggar och saknar klyföppningar. På den ofre (nedåtvända) sidan finnas ytterst talrika klyföppningar, hvilka äro strödda öfver hela ytan.' I have examined a very large number of specimens from the Botanic Gardens of Kew, Strassburg, Glasgow, &c., and in all cases I found that the dorsal leaves, as in the case of the ventral leaves, bear stomata on both sides.

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[1] Beiträge zur Morphologie, Anatomie und Biologie der *S. lepidophylla*, Spr. [illegible]

Erikson's monograph[1] deals in considerable detail with the leaf-structure in the Lycopodiaceae. The author has examined thirty species of *Selaginella*, apparently chiefly from herbarium material; of these I have examined twenty. His work, to a large extent, covers the same ground as my own; still, as I have had opportunities of studying thirty-two species which Erikson has not had access to, and as I am able to supplement to some extent many of his observations, and to add several points which he has not touched, I may venture to consider his memoir as not rendering the publication of the present paper altogether unnecessary. Into the details of his work I need not at present go, but merely give, for convenience of comparison, his scheme of classification of the species according to their leaf-structure. Erikson distinguishes three types of leaf: the first division includes such as have leaves with the upper epidermal cells conical and with, at the same time, a homogeneous spongy mesophyll, e.g. *S. Martensii*, &c.; the second group includes those which have leaves provided with a distinct palisade-layer, some of these, like *S. Kraussiana*, with upper and under epidermal layers similar, others, like *S. Wildenowii*, with upper epidermis different from the under. The third group have leaves without palisade-layers, again subdivided into those with a similar epidermis above and below, e. g. *S. spinosa*, and those with epidermis above and below dissimilar, e. g. *S. serpens*. In comparing Erikson's scheme with that of Dangeard, it will be seen that the former takes the presence or absence of a palisade-layer as the basis of primary division, and subdivides the groups according to whether the epidermis of the upper side is like that of the lower side or unlike; whilst Dangeard selects the character of the epidermis as his primary basis of classification, and subdivides according to the nature of the mesophyll. It seems to me quite immaterial which plan is adopted, although Dangeard's method is perhaps the simpler of the two. It has the further advantage of being based on a somewhat more constant character, although neither the nature of the epidermis nor the composition of the mesophyll, singly, seems to me to afford a perfectly satisfactory basis. In the order of description of the species I have therefore combined these two characters. The great majority of the species which I have examined (thirty-two out of fifty-three) have the epidermal layers different in character, and show in section a reticulate mesophyll without any very definite palisade-layer. Three additional species with very delicate leaves have practically no mesophyll, but possess the epidermal characters of the same nature as those of the majority. The remaining species have the upper and under epidermis practically alike; some, however, of these have, and others have not, a distinct palisade-layer. I have examined several hundred leaves, and find scarcely one of the characters I have mentioned at all constant. Even on the same epidermal surface, one finds elongated cells in one part and quite short polygonal cells in another. Again with regard to the mesophyll, many species show a quite distinct *tendency* to form a palisade-layer (though not a compact one) where, on the whole, one would consider the mesophyll to consist of reticulate parenchyma only. As for the stomata, these are most variable in their distribution, being situated sometimes on the ligular, sometimes on the aligular, and sometimes on both surfaces; sometimes over the midrib only, sometimes on the wings, sometimes generally all over the surface.

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[1] Bidrag till Kännedomen om Lycopodinebladens Anatomi: Arbet f. Lunds Bot. Instit., 1892.

In the arrangement of the present paper I purpose first to give a brief account of the peculiarities of the leaves of each species, and thereafter to add one or two notes on special points, such as the structure and development of the stomata and the histology of the vascular bundle. I had occasion in a recent paper[1] to draw attention to the necessity of considering the internal anatomy in the classification of the very numerous species. I was led from a study of the stem-structure to arrange the species somewhat differently from the scheme adopted by systematists. It will be interesting finally to see in how far the anatomy of the leaf supports or refutes the views expressed in that paper.

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[1] On the diagnostic characters of the subgenera and species of *Selaginella*, Spr.: Trans. Biol. Soc. Liverpool, 1893.

SECTION I. ANATOMY OF SPECIES.

A. Martensii-Type.

All the species coming under this heading have the epidermis on the ligular and aligular surfaces of the leaves dissimilar, and a mesophyll consisting of reticulate parenchyma. (It will be understood that, to save repetition, the chief differences in histology will alone be pointed out. Unless otherwise stated, the characters in the various species are those described in the type-species.)

1. **S. Martensii**, Spr. Pl. ix, Fig. 3.

Ventral leaf. The leaves are lanceolate, somewhat elongated, and unequal-sided with narrow, bluntly pointed apices. The margin consists of elongated sclerotic fibres, from which numerous unicellular trichomata arise. The cells of the ligular epidermis are polygonal, wavy on surface view, conical in section. No stomata occur on this face. The aligular face consists chiefly of elongated plate-like cells with sinuous margins, amongst which occur scattered sclerotic fibres. The stomata are in several rows over the midrib, and the epidermal cells are often sclerotic in the neighbourhood. Occasionally stomata occur on the lamina, near the margin of the leaf. Near the stomata the epidermal cells are considerably shorter. Cuticular warts occur on the surface of the marginal cells.

Dorsal leaf. The leaves are ovoid oblique with well-marked apices, and the margins bear numerous unicellular trichomata. The ligular epidermis consists of elongated, wavy-bordered cells without stomata. The aligular surface consists of polygonal sinuous cells, peg-shaped in section, with numerous stomata. Very many of these stomata have their guard-cells surrounded by strongly thickened epidermal cells, especially on the walls touching the guard-cells (Fig. 3).

Section. (Unless otherwise stated, the section described is that of the ventral leaf.) On the ligular face the cells are cuticularized and obconic, and rest on the lower or aligular epidermis at the margins, but are separated from it in the thicker regions of the leaf by reticulate mesophyll.

2. S. grandis, Moore.

Ventral leaf. The leaves are long, rhomboidal, and sharply acuminate, on the whole equal-sided, and bear shorter or longer spines on the base only. The ligular epidermis consists of polygonal cells, becoming more elongated and wavy towards the margins. A few stomata occur on this face. The aligular epidermis consists of elongated sinuous cells, becoming shorter over the midrib and having scattered sclerotic warty fibres. The stomata are in several rows over the midrib.

Dorsal leaf. The leaves are ovoid pointed oblique with long spiny apices. The ligular face bears no stomata, though these are numerous on the aligular surface. Epidermis as in the type.

Section. As in *S. Martensii.*

3. S. Vogelii, Spr.

Ventral leaf. Leaves triangular ovoid, with blunt apices and without marginal spines. Stomata occur on both ligular and aligular surfaces, though more numerous on the latter.

Dorsal leaf. Leaves oblique ovoid, small, with long apices and without marginal spines. Stomata occur on the aligular surface only.

Section. As in the type.

4. S. haematodes, Spr.

Ventral leaf. Leaves oblique, ovoid, acute, faintly spiny on the outside margin. The polygonal cells of the ligular face become long and wavy bordered nearer the margins; no stomata. The walls of the aligular epidermal cells have irregular thickenings similar to those figured as occurring in *S. producta* (Fig. 6). The stomata are surrounded by similar cells, and warty sclerotic fibres occur among the epidermal cells.

Dorsal leaf. Leaves ovoid, oblique with a long cusp; margin faintly spiny. The ligular epidermis has no stomata, but has the same irregular thickenings that occur in the cells of the aligular epidermis of the ventral leaf. The aligular epidermal cells are polygonal, becoming longer towards the margins. The stomata are surrounded by irregularly thickened cells.

Section. As in the type.

5. **S. erythropus**, Spr.

Ventral leaf. Ovate, oblique, curved, with numerous marginal trichomata with red contents. Stomata occur near the base on the outer region of the ligular epidermis. The elongated cells of the aligular face are not wavy, the cells near the stomata becoming polygonal.

Dorsal leaf. Similar in form to the ventral leaf, but with a long cusp. The ligular face bears numerous stomata distributed over the outside half of the leaf. The marginal cells are sclerotic and warty. The cells of the aligular face are long at the margins, shorter near the midrib where the stomata occur.

Section. The cells composing the reticulum are shorter and more rounded than in the four preceding species.

6. **S. caulescens**, Spr.

Ventral leaf.. Ovate, curved, pointed, with marginal unicellular spines. Stomata occur near the base of the ligular face of the leaf. The cells of the aligular surface are elongated, but not wavy, though warty. The aligular face bears numerous stomata.

Dorsal leaf. Similar in form to the ventral leaf, but with a longer cusp. Stomata occur on the aligular face only.

Section. As in the type.

In var. *argentea* there are no stomata on the ligular surface of the ventral leaf. In var. *japonica* and var. *minor* the ligular surface of the ventral leaf has stomata near the base, and the epidermal cells are elongated on one side of the midrib and polygonal on the other. On the aligular face of the dorsal leaf in these varieties a few sclerotic cells occur on either side of the midrib. I draw attention to these small histological differences by way of emphasizing the great variation that occurs in the leaf-characters.

7. **S. Griffithii**, Spr.

Ventral leaf. Unequal-sided, awl-shaped, pointed, spiny. Marginal sclerotic warty cells on the gibbous side. A few stomata occur on the ligular face near the margin of the leaf. Numerous stomata and sclerotic fibres on the aligular surface.

Dorsal leaf. Ovate, with long cusp; margins warty and spiny. Sclerotic fibres occur among the ligular epidermal cells, though no stomata are present on this face. These are, however, numerous on the aligular face.

Section. As in the type.

8. **S. Karstenian**a, A. Br.

Ventral leaf. Leaves large, very oblique ovoid, blunt apically and with basal wings, very faintly spiny. The polygonal cells of the ligular surface are more elongated and wavy towards the margins; stomata are absent. Sclerotic warty fibres occur on the aligular surface. The stomata are very peculiar. Frequently the walls of the guard-cells are so much thickened as to almost or quite obliterate the lumina, as I have figured in *S. concinna* (Fig. 7). The surrounding epidermal cells are also greatly thickened. It is difficult to see how these stomata can serve any physiological purpose, at least in the mature leaf. I may say in this connexion that I invariably examined leaves taken from several parts of the plants studied, avoiding the very young leaves near the apex, and the old leaves on the basal regions of the stem.

Dorsal leaf. Leaves ovate pointed and faintly spiny. Stomata occur both on and near the margins. The stomata on the aligular face were in many cases sclerotic, as in the case of the ventral leaf.

Section. As in the type.

9. **S. suberosa**, Spr. (Fig. 19.)

Ventral leaf. Ovate-lanceolate, bluntly pointed with spiny margins. The margins have warty sclerotic cells every here and there, except where marginal stomata occur. Warty sclerotic fibres also occur among the aligular epidermal cells, the stomata being confined to the region over the midrib.

Dorsal leaf. Ovate with backwardly directed wings, spiny sclerotic margin, and a long cusp. No stomata occur on the ligular face. The sclerotic fibres on the aligular face are very long, thick-walled, highly refractive, and warty. Stomata occur over the midrib, and a very few at the margins of the basal lobes.

Section. As in the type.

Fig. 19 shows a section of the ventral leaf. The ligular epidermis consists of peg-shaped cells with every here and there sclerotic fibres.

One of these is shown in section. The reticulate mesophyll is of the usual nature common to species belonging to this type. Two warty sclerotic fibres cut across are shown in the aligular epidermis.

10. **S. stenophylla**, A. Br.

Ventral leaf. Oblong-ovoid, bluntly pointed, and slightly spiny. Sclerotic warty fibres occur on the ligular face; warty sclerotic marginal cells alternate with marginal stomata. Similar sclerotic fibres occur on the aligular face, where there are numerous stomata.

Dorsal leaf. The leaf is ovate with a long cusp and spiny margin. The marginal cells are sclerotic, and the leaf has backwardly directed lobes as in *S. Karsteniana*. No stomata occur on the ligular face, though there are numerous sclerotic warty fibres. These also occur on the aligular face. The stomata occur not only over the midrib, but at the margins as well.

Section. As in the type.

The vascular bundle of the leaf of this species will be referred to later.

11. **S. viticulosa**, Klotz. (Figs. 2, 9, 10, 16, 22.)

Ventral leaf. Ovate, oblique, bluntly pointed, with short basal lobes. The margin is formed of elongated pitted cells, from almost every one of which unicellular spines arise. The spines are much shorter near the apex. Stomata occur on the aligular face only, in 4–5 rows over the midrib. Many of these have sclerotic walls (Figs. 9, 10), and not infrequently one meets with abnormal stomata with three guard-cells (Fig. 10); whilst on some leaves I have found the stomata grouped in twos and threes (Fig. 2). All the cells of the aligular surface are elongated and sinuous, and provided with two rows of cuticular warts (Fig. 16). The cells near the midrib are shorter and have less sinuous walls. The cells of the ligular face are wavy and polygonal, but become more elongated towards the margin of the leaf; there are no stomata on this face.

Dorsal leaf. Ovate pointed, oblique, with long cusp. The margin is spiny, and the trichomes are especially long on the basal lobes. Several of the epidermal cells of the aligular surface of the basal lobes bear trichomata. Stomata occur in four or more rows over the midrib on the aligular face; a few also occur on the inside lobe. The stomata are often sclerotic and abnormal, as in the case of the ventral leaf. No stomata occur on the ligular face.

Section. As in the type.

12. **S. serpens**, Spr. (Figs. 4, 14, 29.)

Ventral leaf. Ovoid, blunt, with sclerotic spiny margins (Figs. 4, 14). The cells of the ligular epidermis are polygonal or rounded, becoming elongated with sinuous lateral walls on the basal lobes. The elongated cells of the aligular surface are provided with one row of warts; the cells become shorter near and over the midrib. Stomata occur on this side only.

Dorsal leaf. The leaves are ovoid, cuspidate, very oblique, and the margin is more sclerotic than in the ventral leaf. Stomata occur on both sides—on the aligular side, where they are frequently sclerotic, over the midrib; and on the ligular side on the outside lobe. The elongated cells of the ligular epidermis have 3–4 rows of warts.

Section. As in the type, but the mesophyll-cells are short and closely packed (Fig. 29).

13. **S. cuspidata**, Lk.

Ventral leaf. The leaves are broadly ovate, and cuspidate with a broad marginal band of sclerotic fibres, trichomatous especially towards the base. These marginal cells have no chloroplastids. Stomata occur on the ligular face occasionally, especially on the margins of the basal lobes. The aligular epidermal cells are elongated but not wavy; there are numerous stomata.

Dorsal leaf. The leaves are almost as large as those of the ventral side, and similar in form and margin. Stomata occur on the lamina, but not over the midrib, of the ligular face. Numerous stomata occur on the aligular face.

Section. As in the type.

14. **S. helvetica**, Lk. (Fig. 23.)

Ventral leaf. Leaves awl-shaped, bluntly pointed, unequal-sided, and faintly spiny. The epidermis of the ligular face, which bears no stomata, consists of polygonal cells over most of the surface, but of elongated cells over the basal and apical regions.

Dorsal leaf. The dorsal leaves are nearly as large as the ventral, and of similar shape and histological characters. Stomata occur near and over the midrib on the aligular face, and sparingly also at the edge near the base. On the ligular face stomata also occur over the middle and edge. Their walls are often sclerotic.

Section. The mesophyll consist of rows of elongated cells, which curve upwards from the ventral to the dorsal surface (Fig. 23).

15. **S. denticulata**, Lk.

Ventral leaf. Ovate, somewhat cordate, faintly spiny. Stomata occur on the aligular surface only, over the midrib and wings; a few occur near the margins.

Dorsal leaf. Similar in shape to the ventral, but smaller; faintly spiny. Stomata occur on both sides.

Section. As in the type.

16. **S. patula**, Spr. (Fig. 11.)

Ventral leaf. Lanceolate, oblique, abruptly pointed, with marginal trichomata at the base, spiny at the apex. Stomata occur along the edge of the ligular surface where the ordinary polygonal cells of this surface become longer. The aligular cells (Fig. 11) are partly sclerotic, and each bears one or two rows of cuticularized warts. The cells are shorter over the midrib where the stomata occur.

Dorsal leaf. Leaves ovate, cuspidate with marginal trichomata. Stomata occur on the ligular face near the base. The aligular epidermal cells are warty.

Section. As in the type.

17. **S. convoluta**, Spr.

Ventral leaf. Oblique sickle-shaped, acute, with spiny margins; the lamina near the margins one cell thick. The aligular surface only bears stomata.

Dorsal leaf. Ovate, oblique, acute, with spiny margin. Both ligular and aligular surfaces bear stomata. The cells of the aligular face are, in most parts of the leaf, nearly as long as those of the ligular surface.

Section. Reticulate mesophyll between the two epidermal surfaces, but the upper cells (ligular) are closely packed and form a pseudo-palisade-layer. Dangeard speaks of stomata on the ligular as well as on the aligular face of the ventral leaf. As I have above stated, this is true of the dorsal leaf (which Dangeard does not refer to in this relation), but I have been quite unable to find stomata on the ligular face of the ventral leaf.

18. **S. flabellata**, Spr.

Ventral leaf. Oblique ovate, bluntly pointed, faintly spiny along one side. On both ligular and aligular surfaces the epidermal cells vary in length.

Dorsal leaf. Similar in shape to, but one-third the size of, the ventral leaves. The stomata which occur only on the aligular face are frequently sclerotic.

Section. As in the type. Dangeard's figure of the transverse section of the leaf (*l. c.*, Plate XI, Fig. 20) shows no intercellular spaces. This is quite incorrect.

19. S. producta, Bak. (Figs. 5, 6.)

Ventral leaf. Cordate, oblong-lanceolate, unequal-sided, with sclerotic margins, faintly spiny at and near the base. The aligular epidermis consists of the usual elongated wavy-bordered cells, with many sclerotic fibres interspersed. The stomata are almost all sclerotic (Fig. 5), the lumina of the surrounding epidermal cells being sometimes almost occluded. The epidermal cells also have numerous irregular thickenings on the lateral and end walls (Fig. 6).

Dorsal leaf. Similar to those of the ventral side, but cuspidate. The aligular and ligular epidermal surfaces are similar to those of the ligular and aligular surfaces of the ventral leaf respectively. The stomata, which occur on the aligular face only, are sclerotic.

Section. As in the type, save that the mesophyll-cells are very long.

20. S. bisulcata, Spr.

Ventral leaf. Oblique, ovate, bluntly pointed with spiny margin. The ligular epidermis bears numerous marginal stomata from base to apex. The margin has a warty sclerotic border. Stomata occur over the entire aligular surface.

Dorsal leaf. Oblique, ovoid, cuspidate with spiny sclerotic margins. Stomata occur only on the aligular surface.

Section. As in the type.

21. S. uncinata, Spr. (Figs. 12, 13.)

Ventral leaf. Leaves oblique, oblong, subsessile, with quite smooth (non-hairy) but sclerotic margins. Stomata occur on the aligular face only, the guard-cells having two large chloroplastids in each.

Dorsal leaf. Ovate, with smooth sclerotic margin. Stomata on the aligular face only.

Section. As in the type.

The development of the stomata is discussed in Sect. II.

22. S. plumosa, Bak. (Fig. 21.)

Ventral leaf. Oblong pointed, spiny but non-sclerotic margins. Several stomata occur on the outside marginal region of the ligular face as well as over the midrib on the aligular face. ,

Dorsal leaf. Broadly ovate and suddenly acuminate with spiny margins. No stomata occur on the ligular face.

In *section* the leaf of the species approaches that of *S. Wildenowii*, the peg-shaped ligular epidermal cells being followed by a layer of small cells with large chloroplastids—a pseudo-palisade-layer—followed in turn by a reticulum of larger rounded cells, abutting on the aligular epidermis (Fig. 21).

23. S. Douglasii, Spr. (Fig. 1.)

Ventral leaf. Ovate, oblique, bluntly pointed, with a few hairs on the basal lobes. The spines are commonly sheathed at the base by marginal leaf-cells. The margin is not sclerotic, but the cells are warty. Stomata occur on both faces, over the outside of the basal lobe on the ligular face, and over the midrib on the aligular face.

The *dorsal leaf* is similar in shape with a cusp provided with hooked spines. The margin is similar to that of the ventral leaf. Stomata occur on both surfaces.

In *section* the leaf shows a reticulate mesophyll only—there are no palisade-cells.

Both of these species (22 and 23) show a tendency to have the ligular and aligular epidermis composed of somewhat similar cells, and thus form a transition to types B and C.

The succeeding ten species conform in the structure of their leaves to the *Martensii*-type, although their stem-anatomy shows a distinct advance, having three or more steles instead of one.

24. S. inaequalifolia, Spr.

Ventral leaf. Oblique awl-shaped, bluntly pointed, with sclerotic faintly spiny margins. The axillary leaf resembles the ventral, save that the lamina is equalized. Stomata occur on the aligular face plentifully, and a few also occur near the margin on the ligular face.

Dorsal leaf. Similar in shape to the ventral leaf, but narrower. Stomata occur on the aligular face only.

Section. Similar to that figured for *S. Wildenowii* (Figs. 30, 31).

25. **S. Wallichii**, Spr.

Ventral leaf. Oblong oblique, bluntly pointed, without marginal spines. Stomata occur on the margin on the ligular face, as well as abundantly on the aligular surface.

Dorsal leaf. Similar in shape but cuspidate. Stomata occur on both ligular and aligular surfaces.

Section. As in *S. Wildenowii.*

26. **S. Wildenowii**, Bak. (Figs. 30, 31.)

Ventral leaf. Oblong, unequal-sided, with crenate non-spiny margins. Stomata occur on the aligular face only.

Dorsal leaf. Oblong, unequal-sided, cuspidate. Stomata distributed over the aligular face only.

Section. The ligular epidermis in section is composed of obconic cells with one or two large chloroplastids in each, having their truncated apices continuous with a reticulate mesophyll. The aligular epidermal cells are much deeper in the centre than where they touch each other. The walls of the guard-cells are frequently thickened.

27. **S. canaliculata**, Bak. (Fig. 20.)

Ventral leaf. Awl-shaped, or rhomboid with a sclerotic margin and a few spines near the apex. A few stomata occur near the margin of the ligular surface, as well as abundantly on the aligular face.

Dorsal leaf. Similar in shape but cuspidate. Stomata occur on the aligular face only.

Section. As in *S. Wildenowii*, save that in most of the lamina the reticulate mesophyll is wanting, and the ligular peg-shaped cells rest directly on the aligular epidermis (Fig. 20).

28. **S. Mettenii**, A. Br.

Ventral leaf. Oblong, obtuse pointed, with sclerotic faintly spiny margins. A very few stomata occur along the margin of the ligular face. Stomata are generally distributed over the aligular surface.

Dorsal leaf. Ovate, oblique, with sclerotic non-spiny margins. The stomata occur on the aligular face only.

Section. The vascular bundle runs the whole length of the leaf. The mesophyll cells are very short.

29. **S. Lobbii**, Moore.

Ventral leaf. The shape of the ventral, axillary, and dorsal leaves is that of the leaves of *S. inaequalifolia.* The margin of the ventral leaf is sclerotic but not spiny. Stomata occur on the aligular face only.

Dorsal leaf. Stomata distributed chiefly over the lamina on the aligular face—absent from the ligular epidermis.

Section. As in *S. Wildenowii.*

30. **S. gracilis**, Moore.

Ventral leaf. Bluntly pointed, ovate or hastate with the bases produced, that on the side next the stem uncinate. A few stomata occur on the ligular face near the margins. The edges are smooth and sclerotic.

Dorsal leaf. Similar in shape, cuspidate. Stomata occur on the aligular surface only.

Section. As in *S. Wildenowii.*

31. **S. viridangula**, Spr.

The leaves of this species are oblong oblique, the dorsal leaf cuspidate. The *dorsal leaf* has stomata on both ligular and aligular surfaces, but the *ventral leaf* has stomata on the aligular surface only.

Section. As in *S. Wildenowii.*

32. **S. chilensis**, Spr.

Both *ventral* and *dorsal* leaves are lanceolate, almost equal-sided and with produced bases. Both have marginal sclerotic cells, but the margins are only very faintly spiny. A few stomata occur on the ligular face of the ventral leaf near the margin, but none occur on the same face of the dorsal leaf. In *section* both leaves conform to the type described for *S. Wildenowii.*

33. **S. Victoriae**, Moore.

Ventral and *dorsal* leaves are both oblique sickle-shaped, with blunt apices, sclerotic margins, and without marginal spines or hairs. The stomata, which occur only on the aligular face of the ventral leaf, are frequently clustered. Stomata occur on both sides of the dorsal leaf.

Section. The ligular epidermal cells are long, narrow, and closely packed, and merge into the reticulate mesophyll through an intermediate semi-palisade-layer.

The three following species have delicate leaves, and whilst retaining the characters of the species already described in so far as the epidermis is concerned, yet differ in having little or no mesophyll save in the immediate vicinity of the vascular bundle. Hence the ligular peg-like cells come to rest directly on the elongated epidermal cells of the aligular surface. In this respect a section of the leaf resembles that of *S. canaliculata* (Fig. 20), a species which might be considered as forming a transition to this section.

34. **S. molliceps**, Spr.

Ventral leaf. Ovoid but unequal-sided, delicate, with long warty sclerotic marginal cells, and spines which are especially long on the outer margin. Stomata occur over the midrib and along the margin, but are absent from the ligular face.

Dorsal leaf. Ovoid, but not so unequal-sided, and cuspidate, with spiny sclerotic margin. Stomata occur all round the margin and on the aligular face.

Section. The obconical ligular cells rest directly on the aligular epidermis.

35. **S. apus**, Spr.

Ventral leaf. Oblique, unequal-sided, acuminate, with a faintly spiny margin. Stomata occur on the aligular surface as well as near the margin.

Dorsal leaf. Oblique, tapering, slightly spiny. Stomata occur on the ligular surface near the margin, and occasionally actually on it. The aligular epidermal cells are warty, and stomata occur all over the surface.

Section. As in *S. molliceps*.

36. **S. albonitens**, Spr.

Ventral leaf. Lanceolate, acuminate, unequal-sided, faintly spiny along the margin. The polygonal cells of the ligular face have interspersed long sclerotic cells with stomata along the margin. Similar sclerotic cells occur among the elongated cells of the aligular face. Stomata are distributed over the entire surface, and a few occur on the margin itself.

Dorsal leaf. Ovate, with long pointed apex. The margin is spiny and warty. The ligular face is composed of elongated warty cells, some of them sclerotic, but bears no stomata. The aligular surface is composed of elongated wavy cells with interspersed sclerotic fibres and stomata over the entire surface, but not actually on the margins.

Section. As in *S. mollice ps.*

I have already drawn attention to the variation in length and form of the epidermal cells in species with leaves of the *Martensii*-type, but one may say in general terms that the preceding thirty-five species have the ligular surface of the ventral leaf and the aligular of the dorsal leaf composed of polygonal cells, whilst the aligular surface of the ventral and ligular of the dorsal leaf are formed of cells obconic in section and with polygonal bases. In the succeeding species the epidermal layers on the ligular and aligular faces are approximately similar, and the cells are all elongated.

B. Braunii-type.

Three species come under this heading, viz. *S. Braunii*, *S. concinna*, and *S. Bakeriana*. All agree in that the epidermis of both the ligular and aligular surfaces consists of elongated sinuous cells, and in having a distinct palisade-layer as well as reticulate mesophyll. Indications of a palisade-layer in species of the *Martensii*-type have already been noted.

37. **S. Braunii**, Bak.

Ventral leaf. Ovoid with crenate or faintly spiny margins. The cells of the ligular face are elongated, verging to polygonal, whilst those of the aligular face are short with wavy margins. Numerous stomata occur on the aligular surface.

Dorsal leaf. Lancet-shaped, pointed, but without spines on the margin. The ligular epidermis, which bears stomata, is composed of short wavy margined cells, whilst the aligular surface is covered by polygonal or slightly elongated cells, with numerous stomata over the midrib.

Section. As in *S. concinna.*

38. **S. concinna**, Spr. (Figs. 7, 24, 25.)

The leaves are somewhat peculiar in shape. The *ventral leaf* is ovate but very unequal-sided, and has two long spiny backwardly directed lobes, one or both of which is frequently furcate. The ligular face consists of wavy bordered cells more elongated than in *S. Braunii*, without stomata, whilst the aligular face, composed of similar cells, bears stomata over the midrib, often sclerotic (Fig. 7). The *dorsal leaf* is ovate, and has a strongly sclerotic margin with elongated spiny apex. On the inner side there is a narrow basal lobe projecting backwards along the stem, on the outside the lobe is three or four times as broad. All the cells are warty (as are those of the ventral leaf also). Stomata occur on both faces.

Section. In section the ligular and aligular epidermal cells are seen to be strongly cuticularized and warty (Fig. 25). Beneath the ligular epidermis (of the ventral leaf) there occurs a well-marked palisade-layer of narrow deep cells, followed by a reticulate mesophyll resting on the aligular epidermis. At the margins the cells (Fig. 24) are seen to be entirely sclerotic, without intercellular spaces.

39. **S. Bakeriana**, Bail.

The *ventral leaves* are cordate, bluntly pointed and oblong. A few stomata occur on the margins, but none on the ligular face. Four to five rows of stomata occur on the aligular face. The margin is not sclerotic and very faintly spiny, or quite smooth.

Dorsal leaf. Ovate, pointed, with a faintly spiny margin. Stomata occur on the aligular face only.

Section. Like that of *S. concinna*, but the mesophyll is smaller in amount. A loose reticulum surrounds the bundle.

In the remaining species the epidermis of both the ligular and the aligular surfaces, composed of quite or nearly similar wavy-margined cells, is much elongated in the long axis of the leaf. In one only, *S. Lyallii*, there is a well-marked palisade-layer, usually double, as well as a reticulate mesophyll, which latter is alone, as a rule, present in the leaves of the remaining species. In a few, a pseudo-palisade-layer suggests a transition between the majority and the type just discussed.

C. Galeottii-type.

40. **S. Galeottii**, Spr. (Fig. 8.)

Ventral leaf. Broad, ovate, oblique, bluntly pointed, with a faintly spiny margin and a few long multicellular spines on the edge of the basal lobes. Stomata occur on the aligular face only, the guard-cells of which are very frequently folded outwards into the adjacent epidermal cells (Fig. 8).

Dorsal leaf. Oblique hastate, with backwardly directed basal lobes and numerous multicellular hairs. Stomata occur on the aligular face only.

Section. As in *S. delicatissima.*

41. **S. delicatissima**, A. Br. (Fig. 18.)

Ventral leaf. Ovate oblique, with warty and spiny marginal cells not sclerotic. Stomata occur between the spines along the margin and on the aligular face, but not on the ligular side.

The *dorsal leaf* is similar in shape, and stomata occur over the midrib and outside edge.

Section. The ligular surface (of the ventral leaf) consists of large cells, tabular in section, followed by an ill-defined layer of peg-shaped cells with one to three chloroplastids in each, with their truncated ends continuous with long branched and reticulate mesophyll-cells, each with many small chloroplastids. The cuticle on both surfaces is very delicate (Fig. 18).

42. **S. sulcata**, Spr.

Ventral and *dorsal* leaves of this species are hastate, and bear well-marked basal lobes with a fringe of multicellular hairs. The margins of both leaves are smooth, or only faintly spiny. Stomata occur only on the aligular faces of the dorsal and ventral leaf.

In *section* the pseudo-palisade-layer is absent, but the ligular epidermis is composed of cells resembling in section the peg-shaped polygonal based cells of the ligular epidermis in the leaves of the *Martensii*-type.

43. **S. Kraussiana**, A. Br.

Ventral leaf. Oblique, pointed, unequal-sided, with spiny margins and warty marginal cells. Numerous stomata occur on the margin and on the ligular surface near it, as well as over the midrib on the aligular face.

Dorsal leaf. Oblique, pointed, and unequal sided, in outline like that of a *Begonia* leaf. The margin has short spines. Marginal stomata occur on the ligular face, and also over the midrib on the aligular face.

Section. As in *S. delicatissima.*

44. **S. Poulteri**, Hort.

Ventral leaf. Nearly circular or slightly oblique, slightly pointed, with a faintly spiny margin. Stomata occur along the margin and near it, also on the ligular surface. The aligular epidermis consists of elongated cells with exceedingly wavy margins and with a few sclerotic fibres interspersed.

Dorsal leaf. Oblong oblique with backwardly directed basal lobe and spiny margins. Stomata occur here also on the ligular face near the margin.

Section. As in *S. delicatissima*.

45. **S. rubella**, Moore.

The *ventral* and *dorsal* leaves are somewhat like those of *S. Poulteri* in form. The margins are faintly spiny. Stomata occur on the margin, and along the edge of the ligular face. Warty sclerotic cells and stomata are frequent on the aligular surface.

The *dorsal leaf* is peltate, the basal lobes joining behind the point of insertion of the leaf. There are no stomata on the ligular face, but they occur plentifully on the aligular surface. All the cells of the latter face are warty.

Section. As in *S. delicatissima*.

46. **S. lepidophylla**, Spr.

The *ventral* and *dorsal* leaves are both elongated ovate with delicate tapering apices and of nearly the same size. The cordate bases are fringed with hairs, and the margins are warty and spiny. The cells of both ligular and aligular faces of both leaves are strongly sclerotic. Stomata occur on both ligular and aligular faces of both ventral and dorsal leaves. As already stated (p. 127), Wojinowić and Erikson state that although stomata occur on both sides of the ventral leaf, they are absent from the aligular face of the dorsal leaf. I have examined a large number of dorsal leaves from several plants, and never failed to find stomata both on the ligular and aligular epidermis.

Section. The mesophyll consists of reticulate parenchyma only.

47. **S. involvens**, Spr.

The leaves are rhomboid and fringed with spiny processes. The apices are long and tapering, like those of *S. lepidophylla*. The epidermis of both sides is like that of the preceding species, and stomata occur on both ligular and aligular faces of both dorsal and ventral leaves.

In *section* the leaf resembles *S. helvetica* (Fig. 23), but the cells of the reticulate mesophyll next the ligular face are more closely packed, forming a pseudo-palisade-layer. Distinct emergences occur on the basal lobes, consisting of three, four, or more thick-walled spines, fused together.

48. **S. pilifera**, A. Br.

The leaves of this species are very similar to those of *S. involvens*. The apices are long, and the margins are sclerotic and very spiny. Stomata occur on both ligular and aligular faces of both ventral and dorsal leaves.

The leaf in *section* shows the same characters as that of the preceding species.

The remaining three species belonging to this series differ from those already described in having spirally arranged similar leaves. In structure, however, they closely resemble those of the *Galeottii*-type. All three have strongly sclerotic leaf-cells of dense and firm texture. The leaves are further equal-sided and fringed with well-marked spines or emergences. The reticulate mesophyll is large in amount, and there is no palisade-layer.

D. Spinosa-type.

49. **S. spinosa**, A. B. (Fig. 26.)

The leaves of the erect vegetative axis are approximately similar. The first leaves on the creeping portion of the stem, which are often opposite or nearly so, are ovate acuminate and placed far apart. Their margins are entire, or have one or two multicellular spines. On the erect axis the leaves become more lanceolate and pointed, and have two to four prominent recurved spines on either side. The margins of all the leaves are strongly cuticularized.

The ligular epidermis of the leaf of the prostrate axis is composed of oblong cells with wavy margins. The aligular epidermis is similar in character. Stomata occur on the aligular face only. A few stomata occur along the margin where the cuticularized thick-walled cells are absent.

The epidermal characters of the leaves of the erect stem are similar to those of the leaves of the creeping axis. Stomata occur on the aligular face, rarely along the margins.

In *section* the mesophyll-cells are found to be homogeneous in character, and all are more or less elongated in the long axis of the leaf, forming a loose reticulum. In older leaves the aligular epidermis shows a tendency to separate from the mesophyll (Fig. 26).

50. **S. rupestris**, Spr. (Figs. 27, 28.)

As in the preceding species, the leaves are homophyllous, elongate lanceolate, with long spiny cusps, and margins fringed with unicellular spines. The ligular epidermis consists of elongated wavy cells with warty cuticle. No stomata occur on this face. The stomata on the aligular surface are confined to a groove situated above the midrib.

In *section* the leaf is boat-shaped. A sclerotic hypoderma, one or two cells deep, occurs on the lateral wings beneath the epidermis. The mesophyll towards the aligular surface consists of much elongated branched and anastomosing cells; towards the aligular face the cells are much shorter (Figs. 27, 28).

51. **S. oregana**, Eat.

The leaves in form and histological structure are almost precisely similar to those of *S. rupestris*.

E. Lyallii-type.

52. **S. laevigata**, Bak., var. **Lyallii**, Spr. (Fig. 17.)

As in the case of the stem, this species differs somewhat in its leaf-structure from those already described.

The ventral leaf is ovoid, equal-sided, and bluntly pointed with a smooth margin. The dorsal leaf is more elongated and lanceolate, without marginal spines. Both have descending wings adnate to the stem.

The *ventral leaf*. The edge is composed of elongated thick-walled cells with little chlorophyll. The epidermis of both surfaces consists of elongated rhombic plates with wavy outlines. Several rows of stomata occur on the aligular face, close to and over the midrib, where the epidermal cells are much shorter. There are no stomata on the ligular face. The *dorsal leaf* has quite similar characters.

In *section* (Fig. 17) the ligular epidermis is followed by a very distinct palisade-layer, one or, in many places, two cells deep. The main mass of the leaf is occupied by reticulate mesophyll, absent however at the margins and apex. The vascular bundle consists, in section, of three strands of spiral tracheides, the central strand being surrounded by phloem, and all three imbedded in small-celled parenchyma without intercellular spaces.

SECTION II. COMPARATIVE ANATOMY.

1. *The distribution of the Stomata.*

In comparing the different species, one is struck by the great variation in the distribution of the stomata on the leaf. As has been pointed out in the discussion of the individual species, stomata may occur on the ligular or aligular surfaces, or on both; generally over the midrib, but frequently on the marginal lamellae or even on the margin itself. A glance at the following tabular statement shows that out of fifty-two species examined, fourteen species and one variety have stomata on the aligular face only of both dorsal and ventral leaves; ten species and two varieties have stomata also on the ligular face of the ventral leaves; eight have stomata on the ligular face of the dorsal but not on the corresponding face of the ventral leaf; ten species have stomata on both ligular and aligular faces of both types of leaf; whilst twelve species have stomata actually on the margin either of the dorsal or ventral leaf, or not removed more than one cell from it. One can say therefore, though only in very general terms, that the stomata in the genus are distributed as a rule on the aligular epidermis.

2. *Structure and development of the Stomata* (Figs. 12, 13).

I have followed out the stages in the development of the stomata in several species—e.g. *S. uncinata* (Figs. 12, 13), *S. Kraussiana*, &c.—and find no essential differences between them. The stomata appear to be quite of the normal phanerogamic type, with two guard-cells, each provided with a nucleus and a variable number of chloroplastids, not infre-

STOMATA OCCUR ON THE

VENTRAL LEAF.			DORSAL LEAF.		
Ligular face.	*Aligular face.*	*Margin.*	*Ligular face.*	*Aligular face.*	*Margin.*
	S. Martensii			S. Martensii	
S. grandis	S. grandis			S. grandis	
S. Vogelii	S. Vogelii			S. Vogelii	
	S. haematodes			S. haematodes	
S. erythropus	S. erythropus		S. erythropus	S. erythropus	
S. caulescens	S. caulescens			S. caulescens	
„ var. japonica	„ var. japonica			„ var. japonica	
„ var. minor	„ var. minor			„ var. minor	
	„ var. argentea			„ var. argentea	
S. Griffithii	S. Griffithii			S. Griffithii	
	S. Karsteniana		S. Karsteniana	S. Karsteniana	S. Karsteniana
	S. suberosa	S. suberosa		S. suberosa	S. suberosa
	S. stenophylla	S. stenophylla		S. stenophylla	S. stenophylla
	S. viticulosa			S. viticulosa	
	S. serpens		S. serpens	S. serpens	
S. cuspidata	S. cuspidata		S. cuspidata	S. cuspidata	
	S. helvetica		S. helvetica	S. helvetica	
	S. denticulata		S. denticulata	S. denticulata	
S. patula	S. patula		S. patula	S. patula	
	S. convoluta		S. convoluta	S. convoluta	
	S. flabellata			S. flabellata	
	S. producta			S. producta	
	S. bisulcata	S. bisulcata		S. bisulcata	
	S. uncinata			S. uncinata	
S. inaequalifolia	S. inaequalifolia			S. inaequalifolia	
S. Wallichii	S. Wallichii		S. Wallichii	S. Wallichii	
	S. Wildenowii			S. Wildenowii	
S. canaliculata	S. canaliculata			S. canaliculata	
S. Mettenii	S. Mettenii			S. Mettenii	
	S. Lobbii			S. Lobbii	
S. gracilis	S. gracilis			S. gracilis	
	S. viridangula		S. viridangula	S. viridangula	
S. chilensis	S. chilensis			S. chilensis	
	S. Victoriae		S. Victoriae	S. Victoriae	
S. plumosa	S. plumosa			S. plumosa	
S. Douglasii	S. Douglasii		S. Douglasii	S. Douglasii	
	S. molliceps	S. molliceps		S. molliceps	S. molliceps
	S. apus	S apus	S. apus	S. apus	S. apus
S. albonitens	S. albonitens	S. albonitens		S. albonitens	
	S. Bakeriana	S. Bakeriana		S. Bakeriana	
	S. Braunii		S. Braunii	S. Braunii	
	S. concinna		S. concinna	S. concinna	
	S. Galeottii			S. Galeottii	
	S. delicatissima	S. delicatissima		S. delicatissima	
	S. sulcata			S. sulcata	
S. Kraussiana	S. Kraussiana	S. Kraussiana	S. Kraussiana	S. Kraussiana	S. Kraussiana
S. Poulteri	S. Poulteri	S. Poulteri	S. Poulteri	S. Poulteri	S. Poulteri
S. rubella	S. rubella	S. rubella		S. rubella	
S. lepidophylla	S. lepidophylla		S. lepidophylla	S. lepidophylla	
S. involvens	S. involvens		S. involvens	S. involvens	
S. pilifera	S. pilifera		S. pilifera	S. pilifera	
	S. spinosa			S. spinosa	
	S. rupestris			S. rupestris	
	S. oregana			S. oregana	
	S. Lyallii			S. Lyallii	

quently two only. In some species, e.g. *S. viticulosa*, stomata with three or even four guard-cells may be found, similar to those described and figured by Haberlandt[1] as occurring in the sporogonium of Mosses, and by Farmer[2] in *Iris*. The dermatogenic layer immediately over the midrib undergoes division so as to form a band of variable breadth of almost square cells, at first (i.e. near the base of the young leaf) quite indistinguishable from each other. Presently some of these cells, after division has ceased, become rounded and contain denser protoplasm; these are the mother-cells of the stomata. The dermatogenic cell therefore becomes the mother-cell of the stoma directly, and undergoes division by formation of a vertical wall in the long axis of the leaf. In some cases this wall is oblique. The median wall splits, and a central elliptical pore is formed, as in the normal mode of formation of stomata. In the older leaves of many species the walls both of the guard-cells and of the surrounding epidermal cells become greatly thickened, as, for example, in *S. Martensii*, *S. producta*, *S. concinna*, *S. viticulosa*, &c. The stomata, which are in some species formed directly on the margin, do not appear to differ in any respect from those of other parts of the leaf.

3. *The Epidermal Cells* (Figs. 6, 11, 12, 13, 16, &c.).

Reference has already been made under the individual species to the great variety in length and general form of the epidermal cells. Generally speaking, one may distinguish three types of cell in the superficial layer of the leaf: (1) conical cells, having their somewhat square or more commonly polygonal bases turned outwards, and their truncated ends in continuity with the mesophyll (Fig. 22); (2) elongated, square-ended, or tapering plates, with more or less sinuous lateral walls (Figs. 6, 11); (3) sclerotic, warty, and elongated fibres, which occur along the margin in many species (Fig. 4), or scattered irregularly amongst the epidermal cells of either of the two preceding types (Figs. 11, 16, 19). Even on the same leaf-surface one finds polygonal cells near the stomata over the midrib, whilst on the margins and base the cells are of the elongated type.

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[1] Beiträge zur Anatomie und Physiologie der Laubmoose. Pringsh. Jahrb. XVII. 3. 1886.

[2] Ann. Bot., No. xiii, p. 174.

4. *The Mesophyll.*

The mesophyll varies also very greatly in amount, and also in the form of its component elements. In general the main mass of the leaf consists of a reticulum of longer or shorter cells (compare Figs. 18 and 29) with comparatively large intercellular spaces. In certain species a pseudo-palisade-layer is formed, either of the terminal superficial cells of the reticulum (Fig. 23), or of special smaller cells connected with the reticulum (Fig. 21). In other cases, again, a quite definite palisade-layer is formed, as in *S. concinna* (Fig. 25) and *S. Lyallii* (Fig. 17). Towards the edges of most leaves, and in some cases (e.g. *S. molliceps*, &c.) throughout the entire leaf (save immediately round the vascular bundle), the mesophyll is entirely wanting, and the two epidermal layers are in contact. In such cases the guard-cells of the stomata are very shallow, and since the aligular epidermal cells are deep, a well-marked respiratory and transpiratory cavity is left, even in a leaf only two cells thick

5. *The Vascular Bundle.*

The vascular bundle is almost invariably simple, and extends on an average through five-sixths of the length of the leaf. In some cases it reaches to the base of the cusp, but never so far as I have seen enters it. In one or two cases, e.g. *S. molliceps*, I have found a bifurcate midrib, but this so rarely that I am forced to believe that the bifurcate condition is to be explained as a mere sport.

In structure the bundle is very simple. In *S. Kraussiana*, for instance, the xylem of the midrib near the base and as far as the middle of the leaf consists of four to five delicate tracheides, of which as a rule one is annular, the rest spiral, the spiral being the first to appear. In the upper third of the leaf the protoxylem-elements become accompanied by several short reticulate tracheides which flank the spiral tracheides, and in section (e.g. of *S. Braunii*) may enclose the spiral elements completely. In all cases which I have examined the vein is much more delicate nearer the base of the leaf, and expands at its apex. In the very young leaf of *S. Kraussiana* the reticulate elements at the apex may be fully developed before the differentiation of the characteristic markings on the spiral and annular tracheides. The xylem is partly surrounded by a few long and narrow parenchyma-cells, and one or two sieve-tubes with characteristic plates are present if the bundle be at all massive. In many cases I was quite unable to convince myself that genuine sieve-tubes were present. All the tubes investing the xylem are elongated, thin-walled, and contain nuclei and protoplasm. These again are surrounded by a layer of larger cells without intercellular spaces, usually containing a small amount of chlorophyll, which may be considered as a peridesm. I find no evidence of the existence of a genuine endodermis, save just at the base of the leaf, where cells comparable to and in direct continuity with the endodermal cells of the stele occur. The midrib of *S. Lyallii* is somewhat irregular in character. The xylem-elements are arranged in three patches; the central strand alone has genuine sieve-tubes on its aligular face, but each of the three strands is surrounded by parenchyma, and all three are enclosed in a definite layer (probably peridesm), abutting on reticulate mesophyll below and palisade-cells above (Fig. 17).

In a previous paper[1] I called attention to certain types of stem-structure in the genus *Selaginella*, and made a comparison between the grouping of species based on such anatomical characters and that usually accepted by systematists. Considering the anatomy of the stem only, the majority of species examined may be grouped round *S. Martensii*, all characterized by the presence of a single stele and by dorsiventrality both of external morphological features and internal anatomy. *S. uncinata* formed a transition-type to that of *S. inaequali-*

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[1] On the diagnostic characters of the subgenera and species of *Selaginella*: Trans. Biol. Soc. Liverpool, 1895.

folia: the former shows a distinct tendency to that splitting of the single stele into dorsal, median, and ventral steles, which is carried to its extreme in the latter type. All the species which have the *Martensii*-type of leaf belong to the monostelic and tristelic series as defined in that paper. A few species, also monostelic so far as their stem-structure is concerned, vary somewhat from the normal type of leaf-structure. *S. molliceps*, *S. apus*, and *S. albonitens* present no difficulty, seeing that their leaves differ from the *Martensii*-type only in having the reticulate mesophyll reduced to a minimum. *S. Bakeriana*, *S. Braunii*, and *S. concinna*, on the other hand, differ from the *Martensii*-type in two respects; viz. in having a more or less distinct palisade-layer, and in that the epidermis of the ligular and aligular surfaces is more or less similar. As I have, however, tried to point out above, the length of the epidermal cells is by no means constant even in the same species; and several species belonging to the *Martensii*-type, e.g. *S. helvetica* and *S. plumosa*, form pseudo-palisade-layers, so that these three species are perhaps not so aberrant as they at first sight appear. In the series with quite equivalent epidermis on the ligular and aligular faces, we find all the species with two laterally placed steles, e.g. *S. Kraussiana*, as well as a few monostelic species. Of these latter, three (*S. pilifera*, *S. lepidophylla*, and *S. involvens*) have a peculiar inrolling habit, which may have something to do with the equivalence of the epidermis on both leaf-surfaces, whilst the remaining three (*S. rupestris*, *S. spinosa*, and *S. oregana*) are homophyllous. *S. Lyallii* is peculiar not only in regard to the anatomy of its stem but also in its leaf-structure. Looking at the genus as a whole (at all events the fifty-two species here discussed), one may say that there are three chief types of stem-structure: the dorsiventral monostele, leading to the tristele, and the lateral bistele. The leaf of course is much more likely to be liable to the influence of the environment than the internal anatomy of the stem, and therefore it is less to be depended upon for classificatory purposes. Still it is interesting to note that, with the few not extremely important exceptions noted, all the species with heterogeneous leaf-epidermis belong to the dorsiventral monostelic or allied tristelic types; whilst those with two laterally-placed steles have homologous leaf-epidermis. Without pressing the point too much, one may, I think, say that, on the whole, a classification of these fifty-two species based on the anatomy of the stem is supported by the structure of the leaf.

EXPLANATION OF FIGURES IN PLATE IX.

Illustrating Professor Harvey Gibson's paper on the leaf of *Selaginella*.

Fig. 1. A trichome from the margin of the ventral leaf of *S. Douglasii*, with basal investment of marginal cells. × 350.

Fig. 2. Adjacent stomata of *S. viticulosa*. × 350.

Fig. 3. Thick-walled stomata of *S. Martensii*. × 350.

Fig. 4. Margin of the ventral leaf of *S. serpens*, showing semi-occluded unicellular spines. × 350.

Fig. 5. Stoma of *S. producta*, showing great thickening in surrounding epidermal cells. × 350.

Fig. 6. Aligular epidermis of the ventral leaf of *S. producta*. The cells have localized thickenings. × 350.

Fig. 7. Stoma of *S. concinna*, with almost occluded guard-cells. × 350.

Fig. 8. Stoma of *S. Galeottii*. × 450.

Figs. 9, 10. Stomata of *S. viticulosa*. One is figured with three guard-cells. × 350.

Fig. 11. Aligular epidermis of ventral leaf of *S. patula*. One of the warty sclerotic fibres is shown. × 350.

Figs. 12, 13. Development of stomata from a young dorsal leaf of *S. uncinata*. × 550.

Fig. 14. Marginal spine of *S. serpens*. × 350.

Fig. 15. Multicellular trichome from the basal lobe of the ventral leaf of *S. sulcata*. × 450.

Fig. 16. Sclerotic fibre with two rows of warts from the aligular face of the ventral leaf of *S. viticulosa*. × 350.

Fig. 17. Transverse section of the ventral leaf of *S. Lyallii*. × 350. (For description see text.)

Fig. 18. Transverse section of the ventral leaf of *S. delicatissima*. × 350.

Fig. 19. Transverse section of the ventral leaf of *S. suberosa.* Three of the sclerotic fibres are shown. × 350.

Fig. 20. Transverse section of the ventral leaf of *S. canaliculata* near the margin. × 350.

Fig. 21. Transverse section, ventral leaf of *S. plumosa.* × 350.

Fig. 22. Transverse section, ventral leaf of *S. viticulosa.* × 350.

Fig. 23. Longitudinal section of the ventral leaf of *S. helvetica.* × 350.

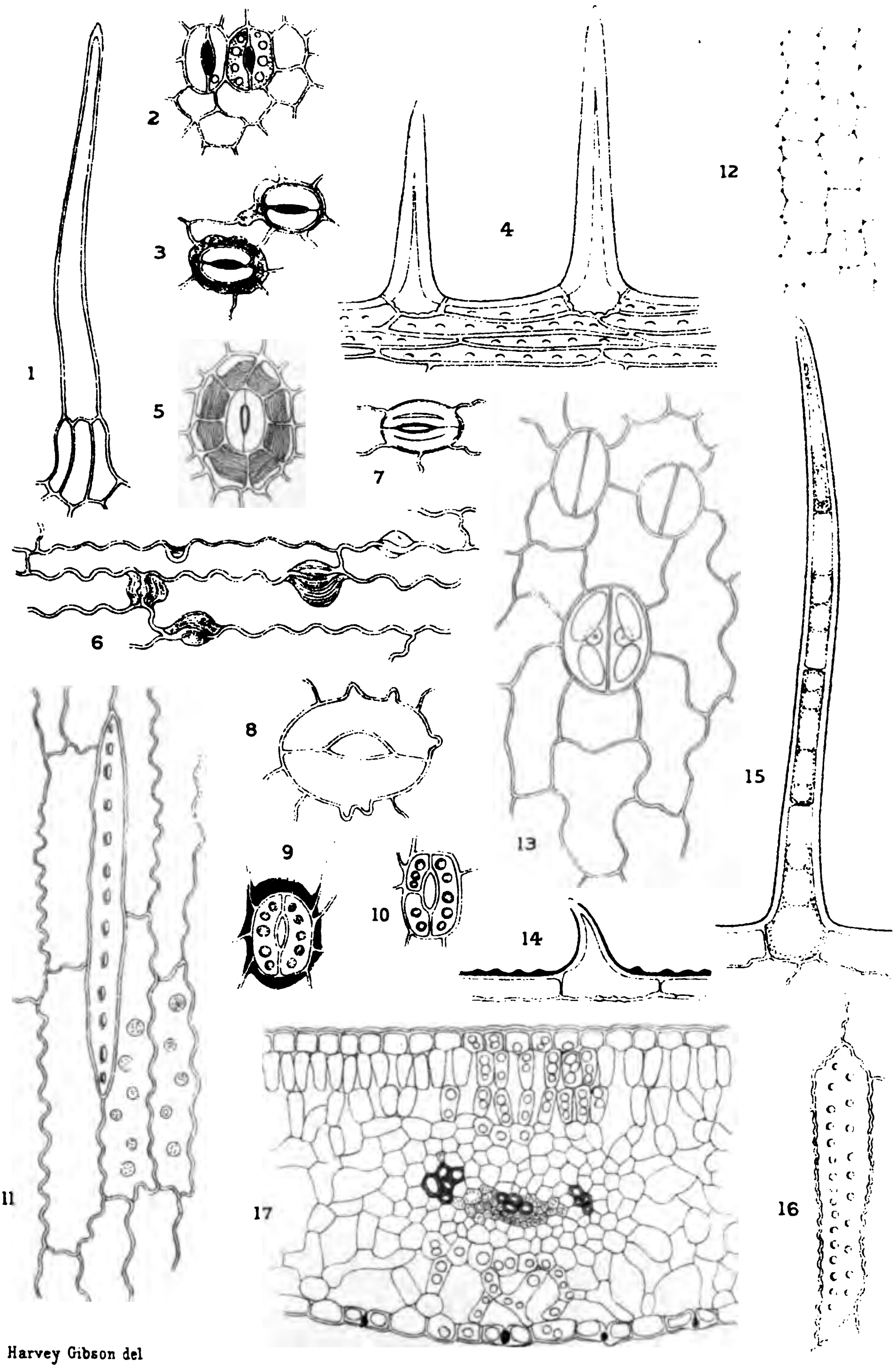
Figs. 24, 25. *S. concinna:* Fig. 24 from the margin of the leaf, Fig. 25 from near the midrib. × 350.

Fig. 26. Transverse section, leaf of *S. spinosa.* × 350.

Figs. 27, 28. *S. rupestris:* Fig. 27, a longitudinal section, × 350; Fig. 28, transverse section, × 75.

Fig. 29. Transverse section of the ventral leaf of *S. serpens.*

Figs. 30, 31. *S. Wildenowii,* Fig. 30 from near the margin; Fig. 31 through the midrib. × 350. The latter section is taken across the upper half of the leaf, where the xylem of the vascular bundle consists chiefly of reticulate elements.



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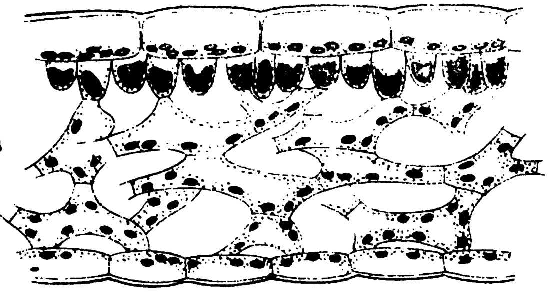
HARVEY GIBSON. — SELAGINELLA.



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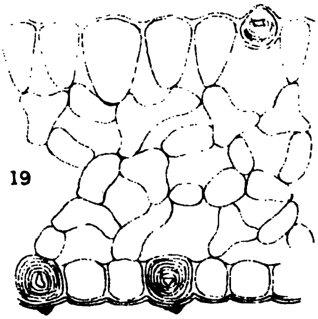

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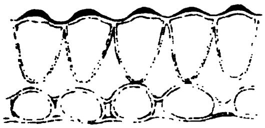

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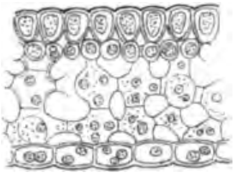

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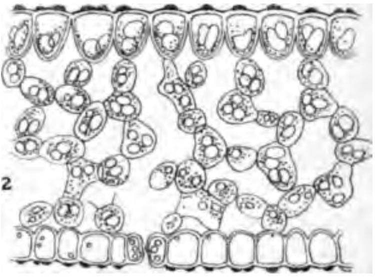

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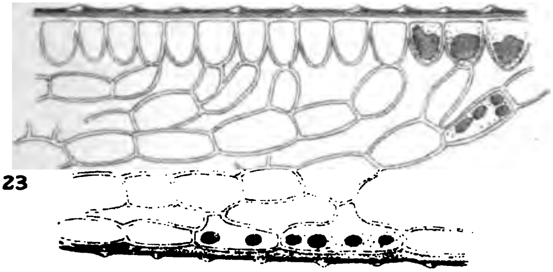

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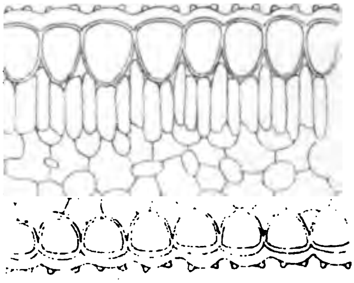

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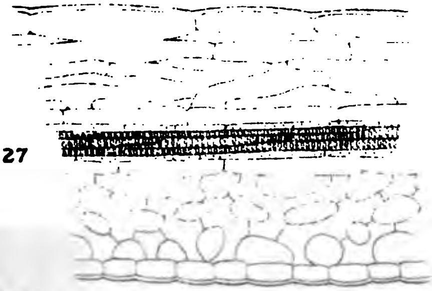

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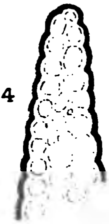

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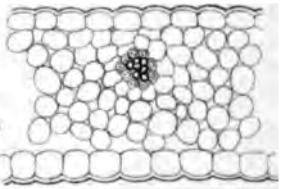

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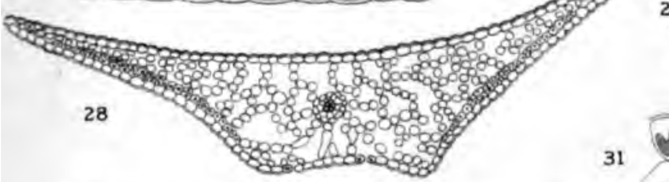

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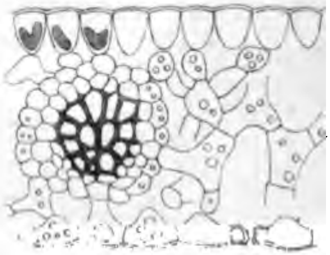

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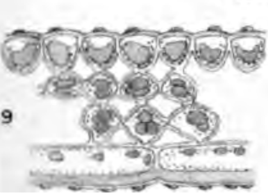

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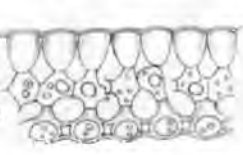

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University Press, Oxford.

NOTES.

PRELIMINARY STATEMENT ON THE DEVELOPMENT OF SPORANGIA UPON FERN PROTHALLI[1]. By WILLIAM H. LANG, M.B., B.Sc.—The observations recorded in this paper were made in the course of an investigation into the relation existing between variability in the Fern plant and apogamy in the prothallus. This research was undertaken at the suggestion of Professor Bower, F.R.S., and has hitherto been conducted in the Jodrell Laboratory, Royal Gardens, Kew. To Dr. Bower and Dr. Scott I am indebted for valuable assistance and advice.

In two of the species investigated, *Scolopendrium vulgare*, L., and *Lastraea dilatata*, Presl., sporangia were borne upon the prothallus. In the former they were sometimes associated with apogamous development of the sporophyte, the details of which differ, however, from previously recorded cases of apogamy. As a considerable period must elapse before an amount of material sufficient for the complete study of details of development can be obtained, it appeared advisable to describe the results obtained from the material at present available. Cultures are about to be commenced in the Glasgow Botanic Gardens for the further study of these abnormal prothalli.

The prothalli of the two species investigated will first be described, and the theoretical bearing of the results briefly considered.

Lastraea dilatata, Presl., var. *cristata gracilis*, Roberts.

The spores from which the cultures of this Fern were made were obtained from a plant in the collection of Mr. C. T. Druery, F.L.S., who kindly supplied me with material. This variety was found wild in Carnarvon in 1870. Spores were sown in the first week

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[1] From the Proceedings of the Royal Society, Vol. lx, 1896.

[Annals of Botany, Vol. XI. No. XLI. March, 1897.]

of November, 1895, upon a carefully sterilized soil, consisting of a mixture of vegetable mould and sand. The pot was kept constantly covered with a glass plate, and the necessity of watering was avoided by standing the pot in a large saucer kept full of water. A close crop of well-formed prothalli, on which antheridia and archegonia were present, completely covered the surface of the soil. In April, 1896, a number of the prothalli bore normal embryos in an early stage of development. Three months later numerous young plants were present, which were found on examination to be normally produced.

The prothalli which had not been fertilized had lost the heart-shaped outline and elongated considerably; some of them reached a length of 2 cm., and were 5 mm. in breadth. The archegonia were very numerous, and were situated upon a distinct cushion, which was continued in the larger prothalli as a well-marked midrib. They were arranged in transverse rows; their necks had opened in a normal manner, and the canal showed the usual brown discoloration. Antheridia were present on some of the prothalli.

In some of these prothalli the midrib was continued into a cylindrical process of variable thickness. This arose in some examples as a direct continuation of the apex, but more frequently was attached to the under surface, just behind the apex of the prothallus; in one instance it was found in a corresponding position on the upper surface. The actual apex usually loses its merismatic appearance; it grows out as a narrow triangular lobe, which consists of colourless cells, and contains tracheides. This lobe closely resembles the 'middle lobe'[1] found in the apogamous prothalli of certain Ferns, and probably corresponds to it. In a few instances this middle lobe is formed, but no cylindrical process arises; in such cases secondary prothalli are produced from the anterior margin of the thin lateral wings, and the whole closely resembles an aborting prothallus of *Aspidium filix-mas* or *Pteris cretica*. When the prothalli are seen from above, the anterior edge can be traced across the base of the cylindrical process. As will be described below, the first sporangia formed on the prothallus are usually situated on this margin, especially on the 'middle lobe.' The process is of the same deep green colour as the midrib. Sexual organs, often in considerable numbers, are borne upon it. They are usually well formed; the archegonia open in the usual manner, and the spermatozoids are capable of active movement when liberated. On other examples variously malformed sexual organs occur. The abnormal archegonia are seated upon small elevations composed of cells which contain chlorophyll; sometimes the neck is open, but other examples have the neck closed and branched. The central cell of the abnormal antheridium is arrested at a more or less early stage of development, while the cells of the wall and the base take on active growth.

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[1] Farlow, Quart. Journ. Microscop. Sc., 1874, p. 268. De Bary, Bot. Zeit., 1878, p. 463.

The sporangia are either isolated or associated together in groups, which bear a striking resemblance to sori. They are borne upon the process or close behind it upon the true middle lobe, and are rarely found upon prothalli which have not produced a cylindrical process. When this is the case, they are always isolated and situated on the edge of a thin continuation of the prothallus arising from the apical depression.

Single sporangia occur frequently on the edge of the prothallus, which, as described above, crosses the base of the process. In a number of examples a single sporangium occupied a median position, and, from earlier stages observed, it is probable that it is to be traced back to the original growing-point of the prothallus. In other cases several sporangia were formed in this region. Isolated sporangia are also found on the process, but more frequently groups are met with. They occupy the upper or lateral faces of the process, and wherever sporangia in early stages of development are found, they are situated at its apex. It is probable that the groups of older sporangia had become displaced from this position by the further growth of the process. The groups were at a considerable distance from each other.

The relative positions of sporangia and sexual organs is a point of some interest, and was not always determined. Archegonia were present close to the sporangia, and at the same level on the process. When the process after producing sporangia had continued its growth, archegonia and [illegible] were produced on the part on beyond the [illegible] [illegible] the other part [illegible] in cases in which more than one group of sporangia had developed, the intervening region bore sexual organs. Rhizoids are also produced abundantly from the [illegible] of the process, and so far as external appearance is concerned there is no reason to doubt the prothallial nature of the region on which the sporangia are situated. The tissue underlying the sporangia, however, presents peculiarities in structure which may modify this conclusion to some extent. Beneath the single sporangia developed on the edge of the prothallus a few tracheides, which agree in every respect with those present in apogamous prothalli, were always to be found. Similar elements were always present in the tissue beneath the groups situated on the process. It is possible that here, as in the case of the sporangia upon the prothallus edge, the first tracheides are developed before the young sporangium can be recognized. All that can be stated with certainty is that they are already present beneath very young sporangia. The tracheides may become connected together into a band, resembling a rudimentary vascular bundle, and suggesting a comparison with the vascular supply of a sorus.

The development of the sporangium could not be followed in detail in the material obtained as yet, but a sufficient number of stages have been found to make it clear that there is no difference of importance from the well-known course of development of the same member on the sporophyte. In the youngest stage seen, the apex of the sporangium was occupied by a tetrahedral cell, the cells destined to form the lateral portions of the wall having already been cut off from a large, dome-shaped terminal cell, the limits of which were clearly recognizable. This was borne upon a stalk cell. A tetrahedral archesporium is formed, from which tapetal cells are cut off. The tapetum subsequently becomes two-layered, and the central cell develops into a group of sporogenous cells. From these, in the most mature sporangia found, a number of dark brown spores had developed, while the tapetum was represented by numerous granules between the spores. The number of spores appeared to be the same as was contained in a sporangium developed on the sporophyte. The sporangium wall was perfectly developed; the cells of the annulus showed the characteristic thickening of their walls, which were of a dark brown colour, and a well-formed stomium was present. When tested with dehydrating agents, the mechanism of the annulus was found to be perfect. The stalk consisted of four rows of cells.

No sporangia have been found in which the spores were ripe, but in view of the advanced stage of development in those observed, there is every probability that some may be obtained. It will be interesting to ascertain if the spores are capable of germination, and if the prothalli produced show any peculiarities. The spores seen already possessed a thick wall on which indications of sculpturing were apparent, and a single nucleus was present in each.

When the unnatural conditions under which they developed are borne in mind, it is not surprising that many imperfect sporangia were found. Such sporangia were in fact the more numerous. Sometimes the arrest of development had taken place before the tapetum had originated from the archesporium; but more commonly the double layer of tapetal cells was present, surrounding a sporogenous cell which had become highly refractive, the nucleus being indistinguishable. The annulus could be made out, but its cells were thin-walled and colourless, and the whole sporangium was pale and more flattened than one of the same age in which sporogenous tissue had formed.

No evidence has yet been obtained of the production of sporophytes, showing vegetative organs, upon the cylindrical process; but one example was seen in which a group of sporangia, situated on the apex of the process, was surrounded by ramenta.

Scolopendrium vulgare, L., var. *ramulosissimum*, Woll.

The cultures of this Fern were made in the manner already described for *Lastraea dilatata*. The spores were obtained from a plant grown in the open air in the Royal Gardens, Kew.

The prothalli were at first heart-shaped, and on many of them normally produced embryos developed. No further changes ensued in those on which young plants were present, and they soon became colourless and died. In those which had remained unfertilized, however, the [illegible] directly into a cylindrical process[1], which was of [illegible] thickness, and in some cases attained a length of 7 mm. The lateral portions of the prothallus showed no further growth and [illegible] brown or colourless appendages to the base of the cylindrical process. On the process were numerous [illegible] further shown by the [illegible] which generally [illegible]. Sections through the process in this stage show that [illegible] and reach almost to the apex [illegible] in the tissue. The archegonia are capable of fertilization, for in some instances normally produced embryos were found.

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[1] [illegible] of them [illegible] are [illegible] the [illegible] further

After the process has in this manner attained a greater or less length, its tip becomes yellowish, contrasting with the deep green colour of the region behind. Near the apex ramenta develop, which soon completely clothe the tip of the process and render it white and conspicuous. Archegonia are present to just below the ramenta. Longitudinal sections at this stage show that one or two small elevations corresponding to the rudiments of the apex of the stem and the first leaf of the sporophyte have been formed. Beneath the broad tip a flat mass of small merismatic cells extends; the merismatic tissue is continuous with that of the stem- and leaf-apices, but, on passing away from these, is separated by several layers of large, non-merismatic cells from the surface. In a slightly older stage the stem-apex has become conical, and a number of leaves have formed which are circinately curved, and formed a bud clothed with ramenta. In the merismatic mass numerous tracheides have been developed. One large group is central in position, and extends to the limit between prothallial and sporophytic tissue, while others are found beneath the bases of the leaves, and are in continuity with their procambial strands. The apex of the stem is occupied by an initial cell, the relation of which to the initial cell or cells of the apex of the process has not yet been traced. The young sporophyte appears to be a direct continuation of the process. It is possible that some of the cases of apogamy recorded by Stange[1] were of this nature, but in *Doodia caudata*, R. Br., which is the only one of his species yet investigated in detail[2], the elevations, from which sporophytes developed, were situated on the under surface of the prothallus. This case appears to be intermediate in character between *Scolopendrium* and the species investigated by De Bary[3].

Several prothalli were found bearing sporangia; these were grouped together in large numbers, usually upon the upper surface of the cylindrical process, but sometimes both above and below. Archegonia were situated close to the groups of sporangia. In the region of the prothallus, underlying the group, a strand of tracheides was found; in one instance this was connected with a spherical mass of tracheides developed to all appearance within the venter of an archegonium whose neck had not opened. The tissue upon which the sporangia are inserted is thin-walled, and its cells have granular contents; it contrasts sharply with the cells of the prothallus which have a large vacuole and walls which stain much more deeply with haematoxylin.

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[1] Ber. der Gesellsch. f. Bot., Hamburg, 1886, p. 43.

[2] Heim, Flora, 1896, p. 329.

[3] Loc. cit.

As in the case of *Lastraea dilatata*, the stages seen render it probable that the sporangia follow the usual course of development. Two layers of tapetal cells are formed which surround a considerable mass of sporogenous tissue. Many of the sporangia fail to attain full development; they remain colourless, and in time wither. A few have been found, however, with a well-developed annulus of a dark colour; these contained spores which have not, however, been examined in detail.

In one case two ramenta overarching a group of sporangia were seen. At first sight it seemed possible that they might correspond to an indusium, but, when taken in connexion with another example in which a cylindrical process, which bore sporangia laterally, terminated in an apogamously produced bud, another explanation appears more probable; this will be referred to again below.

It is worthy of note that another variety of this species has been found to produce young plants, the first fronds of which bore numerous prothalli while still in connexion with the stem[1]. The prothalli on which these plants appeared had been subjected to repeated subdivision, a process which in other species[2] has been found to induce apogamous development of the sporophyte. Unfortunately nothing is known of the manner in which these peculiar plants of *S. vulgare* were produced, but it is possible that they arose apogamously. The case of *S. vulgare* would then be comparable to that of *Trichomanes alatum*[3], in which apogamy and apospory coexist. Prothalli have been found to arise directly from the [illegible] of another variety of *S. vulgare*[4].

An attempt will now be made to bring the peculiar modification of the life-history of these Ferns into relation with previously recorded cases of apogamy and to estimate its theoretical bearing. A full discussion of these points must be deferred until more extended [illegible] have been made.

[illegible] seems [illegible] reason to doubt the prothallial nature of the cylindrical process: its origin, the character of its cells, the presence of functional sexual organs, the development of rhizoids, and the direct transition to an ordinary flat prothallus-apex sometimes met with, are sufficient grounds for this conclusion. The distinction between its origin as a direct continuation of the prothallus, and the cases in which it arises behind the apex which has lost its merismatic character, is not an essential one. Both forms occur in *Lastraea dilatata*; in the latter case the process may be compared with the numerous elevations which appear on the under side of old prothalli of *Doodia caudata*[1], and are capable of apogamous development. The formation of such processes by prothalli which have attained a considerable size without having been fertilized, appears to be of not infrequent occurrence, and is usually associated with apogamy. It is recorded in *Todea pellucida*, Carm., *T. rivularis*, Sieb.[2], and *Athyrium filix-foemina*, Bernh.[3], and the writer has found in *Aspidium frondosum*, Lowe, as many as six apogamous buds, formed from the tips of cylindrical processes which arose from the anterior margin of a prothallus.

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[1] [illegible] Mr. [illegible] read at the Linnean Society, February 23, 1898.
[2] [illegible]
[3] Bower, Ann. of Botany, Vol. i, p. [illegible]
[4] [illegible] 1881.

The term *cylindrical process*[4] has been used to avoid confusion with the middle lobe developed in aborting prothalli of *Pteris cretica* and *Aspidium filix-mas*. This, as De Bary has shown, may be regarded as corresponding to some extent with the first leaf of an apogamous sporophyte[5]. A structure comparable with this middle lobe has been found in prothalli of *Lastraea dilatata*, which had also produced a cylindrical process; usually one or more sporangia were borne upon it.

Tracheides were always present in the tissue beneath sporangia, and the question arises whether their occurrence is to be regarded as of morphological significance. They have been found in the prothalli of a number of species of Ferns, and, in every case investigated, were associated with apogamy. In the case of *Pteris cretica*, the differentiation of the tracheides in the prothallus precedes the origin of the bud[1]. This is the case also with the single sporangia formed on the edge of the prothallus, and probably holds good for the groups of sporangia borne on the process. But tracheides may occur in the prothallus at a distance from the place of origin of buds or sporangia. Putting aside the case of the middle lobe, the prothallial nature of which is open to doubt, a large bundle of tracheides was found in the substance of a fleshy prothallus of a variety of *Scolopendrium vulgare*, which bore numerous archegonia on the surfaces immediately above and below the tracheides. Elongated cells, which resemble sclerenchyma-fibres, occur in the midrib of certain frondose Liverworts[2]. A still more instructive example is afforded by the presence of tracheides in the massive endosperm of certain Cycads[3]. This latter case shows clearly that such elements may be formed in the gametophyte to meet a physiological need. It seems inadvisable, therefore, to lay stress on the presence of tracheides as a means of distinguishing between the two generations, and the more so since their occurrence in a portion of the prothallus which is about to bear a bud or sporangia can be recognized as a physiological advantage. Such means of procuring a sufficient water-supply may be a necessary preliminary to the development of a young sporophyte or a group of sporangia.

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[1] Heim, loc. cit., p. 340, Fig. 12.

[2] Stange, loc. cit.

[3] Druery, Gard. Chron., November 10, 1895.

[4] It is impossible to determine whether the structure to which Wigand (Bot. Zeit., 1849, p. 106) applied this name, and which he inclined to consider as a rudimentary axis, was of the same nature or was a true middle lobe, but the latter appears the more probable conclusion.

[5] Loc. cit., p. 464.

Lastly, it remains to consider the view to be taken of the presence of the characteristic reproductive organs of the asexual generation upon the gametophyte, and to consider its bearing upon the nature of alternation [illegible] in the Archegoniatae. Since the discovery that in certain [illegible] directly from [illegible] of the proper reproductive organs, such cases [illegible] in support of the view that the alternation in the [illegible] On the other hand, it has been [illegible] nature of these [illegible] that the [illegible] generations was a much deeper one [illegible] So [illegible] were [illegible] upon the [illegible] without the intervention of the vegetative organs. At first sight such appears to be the case in the prothalli of the two species described; sporangia were present in close proximity to the sexual organs, the vegetative organs of the sporophyte being, at most, represented by a mass of cells underlying the group of sporangia, and even this distinction may not be recognizable beneath the single sporangia on the edge of the prothallus.

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[1] [illegible] 142.

[2] [illegible]

[3] [illegible]

[4] [illegible]

Several reasons may be adduced, however, against regarding these phenomena as evidence that the alternation of generations found in the Ferns is not antithetic. In the first place, it is to be noted that the two forms in which sporangia have been observed upon the gametophyte are highly variable species, and that the varieties studied were well-marked crested forms. Further, the conditions under which the prothalli existed were in several respects unnatural. Among them the fact that fertilization was prevented by not watering the cultures from above, and that a prolonged growth of the unfertilized prothalli was thereby induced, is of special interest, for it appears that apogamy is liable to occur under such conditions in Ferns which, as a rule, reproduce sexually. While these considerations do not of themselves preclude deductions being made from these peculiar forms of reproduction, they necessitate especial caution in their use in the discussion of broad morphological questions.

Further, a number of reasons exist for considering the production of sporangia on the prothallus as a special case of apogamy. In *Scolopendrium vulgare* a sporophyte may develop from the tip of the cylindrical process. This may happen after a group of sporangia has been developed. In one case two ramenta were present, one on each side of a group of sporangia; they were in every respect similar to the ramenta which develop on the tip of the process when it is being transformed into the apex of a bud. Whenever a group of very young sporangia was seen it was situated upon the apex of the lobe, and the sporangia were in a more advanced stage of development the further the group to which they belonged was removed from the apex. This has been most clearly seen in the case of *Lastraea dilatata*, in which no buds with vegetative organs have as yet been seen, although in one case ramenta were associated with the sporangia, but it also holds for *Scolopendrium*. The explanation of these facts which appears most probable is that each group of sporangia had occupied the apex of the process when very young, and had become further removed from this position as the process continued to increase in length. It is uncertain whether this growth is by direct continuation of the original growing-point of the process, or whether the development of a group of sporangia at the apex necessitates the formation of a new growing-point; possibly both forms occur. If the latter be the case, a process on which several groups of sporangia are present must be looked upon as a sympodium. Some probability is lent to this view by the fact that the first appearance of the process in *Lastraea* is usually as a sympodial continuation of the axis of a prothallus whose true apex has developed one or more sporangia.

Since the group of sporangia and the tissue of peculiar character on which they are seated are developed in the place of an apogamously produced vegetative bud, they may be looked upon as constituting a very reduced sporophyte. The drain upon the resources of the prothallus entailed by the production of this reduced bud, which is incapable of further growth, is much less than when a vegetative bud is formed. This explains why a number of such sporangial groups can be produced and supported by a single prothallus. The occurrence of a number of vegetative buds on a single prothallus is the exception, but may happen, as the case of *Aspidium frondosum*, before mentioned, shows.

It is probable that it is in the constitution of the nuclei that a means of distinction between cells of the oophyte and the sporophyte must be looked for in these cases in which the two generations are in intimate connexion with each other[1].

The complete life history of the Fern is in these cases still further shortened than in the ordinary cases of apogamy; not merely the formation of a zygote by the fusion of antherozoid and ovum, but the formation of an embryo, in which any differentiation of the vegetative organs can be detected, is omitted, and the sporophyte is reduced to a mass of tissue which may be compared to a placenta bearing sporangia. The occurrence of single sporangia upon the edge of the prothallus may, in the light of the series of stages described, be considered as a still further case of reduction of an apogamous sporophyte. While this does not altogether prevent the explanation of the presence of sporangia upon the prothallus from the point of view of the supporters of the homologous nature of the two generations, it brings the present case into line with other exceptions to the normal life-history cycle, whose bearing on the nature of alternation has been discussed by Bower[1]. The present case, although more striking in its appearance, seems, so far as it has been investigated, to afford no sufficient reason for dissenting from the conclusion at which he arrived.

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[1] See Trans. Bot. Soc. Edin., Vol. xx.

It is of interest to note the additional evidence, were such needed, which these observations afford of the generalization made by Goebel[2], that the sporangium is to be regarded as an organ *sui generis*.

From the staff of the Royal Gardens, Kew, I received ready assistance in many practical matters in the conduct of the cultures; my thanks are especially due to the curators, Mr. Watson and Mr. Nicholson.

ON CHEIROSTROBUS, A NEW TYPE OF FOSSIL CONE FROM THE CALCIFEROUS SANDSTONES[3]. By D. H. Scott, M.A., Ph.D., F.R.S.—*The Peduncle.*—The first indication of the existence of the remarkable type of fructification about to be described, was afforded by the study of a specimen in the Williamson collection, from the well-known fossiliferous deposit at Pettycur, near Burntisland, belonging to the Calciferous Sandstone Series at the base of the Carboniferous formation. This specimen is a fragment of stem, of which seven sections are preserved in the collection[4]. Its discoverer thought it might possibly belong to the *Lepidostrobus* found in the same bed. 'If so,' he adds, 'it has been part of the axis of a somewhat larger strobilus than those described[5].'

A detailed examination of the structure of this specimen convinced me that it is essentially different from any Lepidodendroid axis, and is, certainly, a new type of stem[6].

As it was the examination of this fragment of stem which first put me on to the track of the new cone, it may be well shortly to describe its chief characteristics, reserving all details for a future paper.

[1] Annals of Botany, Vol. iv, 1890, p. 347.

[2] Bot. Zeit., 1881, p. 707.

[3] Preliminary Paper, read before the Royal Society, January 21, 1897.

[4] The cabinet-numbers are 539–545.

[5] Williamson, Organization of the Fossil Plants of the Coal-measures. Part III. Phil. Trans., 1872, p. 267.

[6] A short account of this specimen was given by me before the Botanical Section of the British Association at the Liverpool Meeting, 1896. See Brit. Assoc. Report, 1896.

The specimen, which is about 7 mm. in diameter, bears the bases only of somewhat crowded leaves, the arrangement of which, though not quite clear, was most probably verticillate, with from nine to twelve leaves in a whorl, those of successive whorls being superposed. Each leaf-base consists of a superior and an inferior lobe, and each lobe is palmately subdivided into two or three segments.

The leaf-traces, which are single bundles where they leave the central cylinder, subdivide in both planes on their way through the cortex to supply the lobes and segments of the leaf.

The central cylinder is polyarch, the strand of wood having from nine to twelve prominent angles, with phloem occupying the furrows between them. With the exception of the spiral protoxylem-elements at the angles, the tracheae have multiseriate bordered pits, thus differing conspicuously from the scalariform tracheae of the Lepidodendreae. The interior of the stele is occupied by tracheae intermingled with conjunctive parenchyma. There is a well-marked formation of secondary tissues by means of a normal cambium[1].

The Strobilus.

Mr. R. Kidston, F.G.S., kindly informed me that he had in his possession sections of a fossil cone from Burntisland having certain points in common with the Williamson specimen. On inspecting these sections with Mr. Kidston, I was soon convinced that this undescribed cone really belonged to the same plant as the fragment of stem in the Williamson Collection, and that the latter might well be the peduncle of the former. At the same time I satisfied myself, and Mr. Kidston agreed with me, that the whole organization of his cone is fundamentally different from that of any *Lepidostrobus*, the decisive point being that the new cone has compound branched sporophylls, each of which bears a number of sporangia. It became evident that this cone must be placed in a new genus, and the conclusion arrived at from the study of the peduncle was thus confirmed.

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[1] The general structure of this axis, including the course of the bundles and the subdivision of the bracts, is correctly described by Williamson, loc. cit., p. 297. As regards the latter point, he says, 'peripherally the bark breaks up into main or primary bracts, which again subdivide, as in the transverse section, into secondary ones, demonstrating that each primary bract does not merely dichotomize, but subdivides, both horizontally and vertically, into a cluster of bracts—a condition corresponding with what I have already observed in the smaller strobili described.' These smaller strobili are those of the Burntisland *Lepidostrobus*, to which, by a strange coincidence, Williamson, loc. cit., p. 295, erroneously attributed the same character, as regards subdivision of the bracts, which actually exists in the new cone. The only explanation appears to be that Williamson interpreted the structure of the *Lepidostrobus* in the light of that of the peduncle, which, as we shall see, really belonged to a totally different fructification.

Mr. Kidston most generously handed over his sections to me for examination and description, and also obtained for me from the owner the remains of the original block, from which I have had a number of additional sections prepared.

Only a single specimen of the cone is at present known. Before cutting sections, the piece, which includes the base but not the apex of the strobilus, was about two inches long. It was found at Pettycur, near Burntisland, in 1883, by Mr. James Bennie, of Edinburgh. The specimen is calcified, and its preservation is remarkably perfect, so that the whole structure is well shown, though the complexity of its organization renders the interpretation in some respects difficult.

The cone in its present somewhat flattened condition measures about 5 cm. by 2·3 cm. in diameter. The diameter in its natural state would have been at least 3·5 cm. That of the axis is about 7 mm., exactly the same as that of Williamson's peduncle. Thus the extreme length of the sporophylls, which have on the whole an approximately horizontal course, is about 1·4 cm.

The sporophylls are arranged in somewhat crowded verticils, fourteen of which were counted in a length of an inch, 2·5 cm. There are twelve leaves in each whorl, and the members of successive whorls are accurately superposed, a fact which is shown with the greatest clearness in tangential sections of the cone. This is evidently a point of great significance in considering the affinities of the fossil.

The sporophylls themselves have a remarkably complex form. At its insertion on the axis each sporophyll consists of a short basal portion or phyllopodium; the bases of the sporophylls belonging to the same verticil are coherent. The sporophyll branches immediately above its base, dividing into a superior and an inferior lobe, which lie directly one above the other in the same radial plane. Almost at the same point each of the lobes subdivides in a palmate manner into three segments, which assume a horizontal course, whereas the common phyllopodium has an upward inclination. It is probable that sometimes, especially at the base of the cone, there may be two instead of three segments to each lobe. As a rule, however, each sporophyll consists of six segments, of which three belong to the superior (ventral or posterior) and three to the inferior (dorsal or anterior) lobe.

The segments are of two kinds—sterile and fertile. Both alike consist of a long, straight, slender pedicel, running out horizontally, and terminating at the distal end in a thick laminar expansion. The sterile segments are the longer, and their laminae bear, in each case, an upturned foliaceous scale as well as a shorter and stouter downward prolongation[1].

Each of the fertile segments ends in a fleshy laminar enlargement not unlike the peltate scale of an *Equisetum* or a *Calamostachys*. These fertile laminae, which are protected on the exterior by the overlapping ends of the sterile segments, bear the sporangia. Four, perhaps in some cases five, sporangia are attached, by their ends remote from the axis, to the inner surface of the peltate fertile lamina. Each sporangium is connected with the lamina by a somewhat narrow neck of tissue into which a vascular bundle enters. The sporangia are of great length, and extend back along the pedicels until they nearly or quite reach the axis.

The sterile and fertile segments alternate regularly, one above the other, in the same vertical series. So much is evident, but the question which segments are fertile and which sterile, has presented great difficulties, owing to the fact that the same segment can scarcely ever be traced continuously throughout the whole of its long course, and that the pedicels of sterile and fertile segments present no constant distinctive characters. For reasons, however, which will be fully given in a subsequent paper, I think it highly probable that in each sporophyll the segments of the *lower* lobe are sterile, and those of the *upper* lobe fertile, constituting the sporangiophores.

The sporangia and pedicels are all packed closely together so as to form a continuous mass. The external surface of the cone was completely protected by its double investiture of fertile and sterile laminae.

The spores are well preserved in various parts of the cone, and, so far as this specimen shows, are all of one kind, their average diameter being 0·067 mm. At the base of the cone, where macrospores, if they existed, might naturally be looked for, the spores are of the same size as elsewhere. So far, then, there is no evidence of heterospory.

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[1] [illegible] upturned scale and the downward prolongation are [illegible] February 11, 1897.

The spores are considerably larger than the microspores of the *Lepidostrobi*. Those of the Burntisland *Lepidostrobus*, for example, are barely 0·02 mm. in diameter. The spores of our plant approach in size those of *Sphenophyllum Dawsoni*, or the microspores of *Calamostachys Casheana*.

The sporangial wall, as preserved, is only one cell in thickness; it bears no resemblance to the palisade-like layer which forms the wall of the sporangium in *Lepidostrobus*, but has the same structure as that of a *Calamostachys*[1]. The sporangial wall of *Sphenophyllum Dawsoni* is similar.

The anatomy of the axis of the cone agrees closely with that of the peduncle above described, except for the absence of any secondary tissues. The wood has twelve prominent angles, at which the spiral tracheae are situated, so its development was, no doubt, centripetal. The inner tracheae have pitted walls, and are intermixed with scattered parenchymatous cells, imperfectly preserved. The phloem has entirely perished.

The most interesting anatomical feature is the course of the leaf-trace bundles, which can be followed with the greatest exactness on comparing sections in the three directions.

A single vascular bundle starts from each angle of the stele for each sporophyll, and passes obliquely upwards. When less than halfway through the cortex, the trace divides into three bundles, one median and two lateral. The lateral strands are not always both given off exactly at the same point. A little further out the median bundle divides into two, which in this case lie in the same radial plane, so that one is anterior, and the other posterior. The median posterior bundle is the larger, and before leaving the cortex this, in its turn, divides into three. There are now six branches of the original leaf-trace, three anterior, and three posterior, which respectively supply the lower and upper lobes of the sporophyll. The three segments of the lower lobe are supplied by the two lateral bundles first given off, and by the anterior median bundle, while the upper segments receive the posterior median bundle and its two lateral branches. In the base of the sporophyll all six bundles can be

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[1] See Weiss, Steinkohlen-Calamarien, Vol. ii, 1884, Plate XXIV, Figs. 3, 4, and 5; Williamson and Scott, Further Observations on the Organization of the Fossil Plants of the Coal-measures, Part I, Phil. Trans., 1894, Plate 81, Fig. 31.

clearly seen, in tangential sections of the cone, three above and three below. As the segments become free, one bundle passes into each, and runs right through the pedicel to the lamina. In the fertile lamina the bundle subdivides, a branch diverging to the point of insertion of each sporangium.

One of the longitudinal sections passes through the base of the cone, so as to show part of the peduncle in connexion with it. In this peduncle secondary wood is present, just as in the separate specimen belonging to the Williamson Collection. Higher up in the axis of the cone, where the sporophylls begin to appear, the secondary wood dies out. This evidence materially confirms the conclusion that the Williamson peduncle really belongs to our strobilus.

Diagnosis.

It is evidently necessary to establish a new genus for the reception of this fossil; the generic name which I propose is *Cheirostrobus*, intended to suggest the *palmate* division of the sporophyll-lobes (*χείρ*, hand). The species may be appropriately named *Pettycurensis*, from the locality where the important deposit occurs which has yielded this strobilus and so many other valuable specimens of palaeozoic vegetation. The diagnosis may provisionally run as follows:—

Cheirostrobus, gen. nov.

Cone consisting of a cylindrical axis bearing numerous compound sporophylls, arranged in crowded many-membered verticils.

Sporophylls of successive verticils superposed.

Each sporophyll divided, nearly to its base, into an inferior and a superior lobe; lobes palmately subdivided into long segments, of which some (probably the inferior) are sterile, and others (probably the superior) fertile, each segment consisting of an elongated stalk bearing a terminal lamina.

Laminae of sterile segments foliaceous; those of fertile segments (or sporangiophores) peltate.

Sporangia large, attached, by their ends remote from the axis, to the peltate laminae of the sporangiophores.

Sporangia on each sporangiophore, usually four.

Spores very numerous in each sporangium.

Wood of axis polyarch.

C. Pettycurensis, sp. nov.

Cone, 3–4 cm. in diameter, seated on a distinct peduncle. Sporophylls, twelve in each verticil.

Each sporophyll usually sexpartite, three segments belonging to the inferior, and three to the superior, lobe.

Sporangia densely crowded.

Spores about 0·065 mm. in diameter.

Horizon: Calciferous Sandstone Series.

Locality: Pettycur, near Burntisland, Scotland. Found by Mr. James Bennie, of Edinburgh.

Both generic and specific characters are manifestly subject to alteration, if other similar fossils should be discovered. In the meantime the above diagnoses are given in order to facilitate identification.

Affinities.

Any full discussion of affinities must be reserved for the detailed memoir, which I hope to lay before the Royal Society in a short time. At present only a few suggestions will be offered.

The idea of a near relationship to *Lepidostrobus*—so specious at first sight—is negatived by accurate investigation. There may have been a certain resemblance in external habit, as there is in the naked-eye appearance of the sections, but this means nothing more than that the specimen is a large cone, with crowded sporophylls and radially elongated sporangia. The only real resemblance to *Lepidostrobus* is in the polyarch strand of primary wood, but even here the details, as, for example, the structure of the tracheae, do not agree. In other respects the differences from any Lepidodendroid fructification are as great as they can be.

I do not doubt that the genus with which *Cheirostrobus* has most in common is *Sphenophyllum*. The chief points of agreement are as follows:—

1. The superposed foliar whorls. This certainly agrees with the vegetative parts of *Sphenophyllum*, and, according to Count Solms-Laubach, the superposition holds good for the bracts of its strobili also[1].

2. The deeply divided palmatifid sporophylls, agreeing with the leaves of various species of *Sphenophyllum*, e. g. *S. tenerrimum*.

3. The division of the sporophyll into a superior or ventral, and an inferior or dorsal, lobe, agreeing with the arrangement in

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[1] *Bowmanites Römeri*, eine neue Sphenophylleen-Fructification, 1895, p. 242.

Sphenophyllum Dawsoni, or *S. cuneifolium*, according to M. Zeiller's interpretation[1].

4. The differentiation of the sporophyll into sterile segments (bracts) and fertile segments (sporangiophores). The comparison with *Sphenophyllum* is much strengthened if, as I believe to be the case, the segments of the inferior lobe in *Cheirostrobus* are sterile, and those of the superior lobe fertile.

5. The repeated subdivision of the leaf-trace vascular bundles, in passing through the cortex of the axis[2], as in *Sphenophyllum Stephanense*.

6. The attachment of the sporangia to a laminar expansion at the distal end of the sporangiophore. As regards this point, comparison should be made with the *Bowmanites Römeri* of Count Solms-Laubach (loc. cit.).

7. The structure of the sporangial wall.

I think that the sum of these characters, to which others might be added, justifies the suggestion that *Cheirostrobus* may be provisionally placed in the same *phylum*, or main division, of Pteridophyta with *Sphenophyllum*, though indications of possible affinities in other directions are not wanting, and will be discussed on another occasion.

Cheirostrobus, even more than *Sphenophyllum* itself, appears to combine Calamarian with Lycopodiaceous characters, and might reasonably be regarded as a highly specialized representative of an ancient group of plants lying at the common base of these two series.

It appears likely that in *Cheirostrobus* one of those additional forms of Palaeozoic Cryptogams, allowing of comparison with *Sphenophyllum*, has actually been brought to light, the discovery of which Dr Williamson and I ventured to anticipate at the close of our first joint memoir[3].

NOTE ON THE DISCOVERY OF MYCORHIZA. In the Botanische Zeitung for 1886 Wahrlich gives (p. 481) the early bibliography of the discovery of Mycorhiza. He says, 'Schacht was the [illegible] erste, der sie im Rhizom von *Neottia nidus avis*, Rich., fand.' He quotes the first volume of the 'Grundzüge.' The first edition of this, which I have not at hand to refer to, was published in 1842. The second edition, which appeared in 1845, was translated by Dr. Lankester. Schleiden describes (p. 91) 'fibres' which he observed in the cells of the subepidermal tissue of the roots. He concludes by remarking: 'With respect to the real character of these peculiar formations, I have nothing at all to observe.'

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[1] [illegible] Mém. [illegible]

[2] [illegible] Plate XIV, Fig. 2; Plate XV, Fig. [illegible]

[3] [illegible] the Organization of the [illegible] 1894.

An earlier account of the now well-known occurrence of Mycorhiza in *Monotropa Hypopitys*, &c., is by an English botanist, Edwin Lees, and is contained in a botanical journal since extinct, the Phytologist for December, 1841. He gives what seems to me a pretty clear account of the external phenomena:—

'The whole mass (of the roots) is obscured with a hirsuture that appears like a byssoid fungus. These hairy fibres, however, appear to me to be really part of the economy of the plant, imbibing nutriment from the rootlets of the Beech, to which they are closely affixed, and conveying it to the succulent radicles of the *Monotropa*, with which they are also connected' (p. 100).

This paper gave rise to an interesting discussion in which a number of botanists took part, including Wilson the bryologist, and Newman, the accomplished monographer of British Ferns, who had the assistance of Quekett. Newman concludes: 'The byssoid substance . . . I believe to be an intrinsic and most essential portion of the *Monotropa*, and is the part to which I have applied the term root' (p. 298).

In the number for October, 1842, there is an important paper by Thos. E. Rylands, which seems to me to have been entirely overlooked. He arrives at 'the opinion that the "byssoid substance" is really fungoid, and performs no essential function in the economy of the *Monotropa*.' The curious thing is that he examined the roots of other plants, and remarks: 'The *really* fungoid matter found on the roots of Groundsel, *Epilobium*, *Plantago*, &c., had so much resemblance to the substance in question, that it would be difficult by words to render the difference appreciative.' After consulting Mr. Berkeley as to the affinities of the fungus found on *Monotropa*, he describes and figures it as *Lygodesmus Berkeleyi*.

Here the story stops as far as England is concerned. But it seems to me interesting to bring it into notice. It is a good illustration of the fact that accurate observation of even insignificant facts may easily bring one face to face with important and unsuspected discoveries. The time was not ripe for the full appreciation of what those acute inquirers had under their eyes. That required a grasp of the theory of symbiosis which had not arrived.

It is a curious circumstance that on the Continent the battle of priority has already been fought over *Monotropa Hypopitys*. Woronin in 1885 (Bericht. d. D. Bot. Gesellsch. iii. 206) says: 'Alle Prioritätsrechte in der Frage "über die auf Wurzelsymbiose beruhende Ernährung gewisser Bäume durch unterirdische Pilze" müssen demnach nicht Herrn B. Frank, sondern Herrn Fr. Kamienski zugeschrieben werden.'

Kamienski in fact describes the mycelial investment of the roots of *Monotropa* in the Botanische Zeitung for 1881 (p. 459). But his fullest account was given in the following year in a paper, Les organes végétatifs du *Monotropa Hypopitys*, &c., published in the Mémoires de la Société Nationale des Sciences Naturelles et Mathém. de Cherbourg, t. xxiv. He says:—'Toutes les parties les plus actives de la racine . . . sont recouvertes d'une couche épaisse et dense d'un mycélium qui ne permet pas aux racines d'avoir un contact direct avec le sol.'

After minutely describing the mycelium, he proceeds:—'Cette relation étrange entre le champignon et le *Monotropa* n'est pas un fait unique et isolé dans la nature. Nous pouvons le ranger avec d'autres faits semblables auxquels M. de Bary a donné la dénomination de symbiose.'

Drude in 1873 appears also to have observed mycelium in the roots of *Monotropa*, and to have thought it identical with that first observed long ago by Schleiden in *Neottia*. But I have not had the opportunity of consulting his paper.

W. T. THISELTON-DYER.

Postscript. Since the above was in type, I have found that the English authors whom I cite are mentioned in the very complete bibliography of Mycorhiza given by Sarauw in the Botanisk Tidsskrift, Vol. xviii. p. 247, 1892-3. I am indebted to Mr Percy Groom for the reference to this paper.

W. T. T. D.

Notes on some Physiological Properties of a Myxomycete Plasmodium[1].

BY

J. B. CLIFFORD.

With Woodcuts 3, 4, and 5.

WHILE collecting Myxomycetes in woods near Ann Arbor, Michigan, in October, 1895, a quantity of yellow sclerotium of a slime-mould[2] was found and taken to the botanical laboratory for study. In a few days it passed into the plasmodium condition, and since that time continuous experiments have been made relative to its physiological properties. The work on rheotropism and changes induced by varying temperature is measurably complete, and the results so far new as to warrant publication. Work on chemotaxis is in progress, but has not been carried far enough to establish general conclusions.

It was found that the sclerotium can be induced to assume the active plasmodium condition by merely supplying it with abundant moisture. If this is done, the substance may be placed in a warm or cold room, in the dark or in the light, without apparent effect. In every case, about one week

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[1] The work was done in the Botanical Laboratory of the University of Michigan, under the direction of Professor V. M. Spalding.

[2] The species is apparently *Fuligo varians*, Sommerfelt (*Aethalium septicum*), but as the mature form has not yet been obtained in a series of cultures [illegible] this [illegible] cannot be positively asserted.

[Annals of Botany, Vol. XI. No. XLII. June, 1897.]

elapses before spreading begins. The plasmodium will live for an indefinite period, several months at least, if kept sufficiently moist and clean. But if fresh water is not frequently supplied, and the substratum changed, it soon spoils, a certain disagreeable odour and a reddish-brown colour being an indication that this condition is coming on. There is now (July 1) in the laboratory plasmodium which has been spreading since the 5th of January last. For the past three months no food has been given it but water, and it has no doubt been nourished by the microscopic Fungi and Algae growing in the rotten wood upon which the plasmodium has been kept.

RHEOTROPISM.

Concerning rheotropism of plasmodia, the only original work thus far published is that of Strasburger, Jönsson, and Stahl. In 1878, Strasburger[1] discovered that plasmodium will move against a current of water in the substratum. In 1883, Jönsson[2] observed this property, and gave it the name of positive rheotropism, and in the following year Stahl[3] corroborated Jönsson's work and adopted his term. But in none of this work was there an attempt to measure the force of the current against which the plasmodium will move, or to eliminate the possibility of the movement being induced by a search after food contained in the water.

A series of carefully repeated experiments have proven that the plasmodium employed in this study will, when kept at a uniform temperature and removed from the influence of light, constantly advance against a current of distilled water; and furthermore, if two currents, one of distilled and one of hydrant-water, are caused to flow against it, the substance will as often flow against the one as the other.

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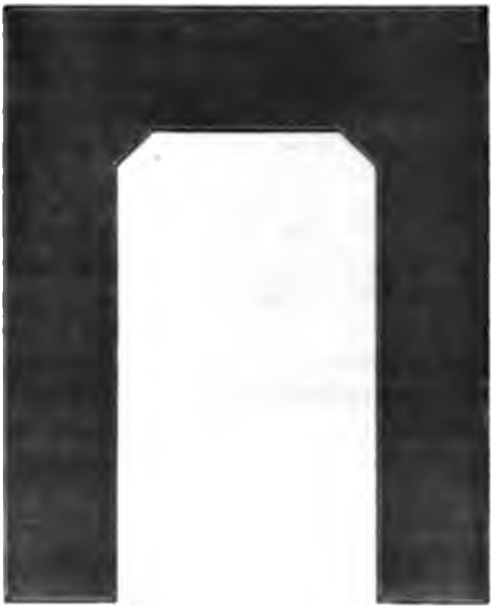
[1] Strasburger, Wirkung des Lichtes und Wärme auf die Schwärmsporen, p. 71, Jena, 1878.

[2] Der richtende Einfluss strömenden Wassers auf wachsende Pflanzen und Pflanzentheile (Rheotropismus), Ber. der deutsch. bot. Ges., Bd. 1, p. 512, 1883.

[3] Zur Biologie der Myxomyceten, Bot. Ztg., p. 144, 1884.

In ascertaining the latter fact, a strip of blotting-paper, cut as indicated in Woodcut 3, had the united lower ends placed in contact with the plasmodium which was spreading upon wood. One arm of this syphon was placed in distilled, the other in hydrant-water, and in repeated trials the plasmodium showed no choice between the two currents, so that, every other factor being eliminated, it can be stated positively that this movement of plasmodium is due to rheotropism alone, and not to substances contained in the water supplied to it.



Woodcut 3.

For the experiments designed to test the force of the current against which the plasmodium is positively rheotropic, wood upon which the substance was creeping was suspended so that the plasmodium just skimmed the surface of the water, which two-thirds filled a glass pan nine inches in diameter. The pan was caused to revolve with uniform rapidity[1], thus creating a current as the water struck the wood, which was placed well to one side. Up to six revolutions per minute the plasmodium moved against the current, but if the number of revolutions was increased to seven it moved with the current, i. e. became negatively rheotropic. If the number was increased beyond seven, it crept up entirely out of the water, as if to protect itself from being swept away.

There is in these movements a remarkable evidence of the extreme sensitiveness of naked protoplasm, exhibiting as it does positive rheotropism up to a certain point, beyond which it would, if continuing this habit, be in danger of being torn from its substratum, and just when the danger-point is reached, being possessed of sufficient sensitiveness to enable it to move out of harm's way. But so unerringly did this power show itself, that there is no doubt of its existence.

THERMOTROPISM.

In conducting the experiments upon thermotropism, the apparatus shown in Woodcut 4 was employed. It consists of a copper tank, 18 in. x 18 in., having upon each of its three sides niches, into which are fitted zinc boxes, so that three-fourths the length of a box projects beyond the tank. Each box is divided into compartments, the first partition passing along a line even with the side of the tank, the second at the same distance from the outer end. Each compartment is provided with openings in the lid, which can be closed by means of corks. The outer one has an opening on the front, near the top. The tank is filled with water, and heat applied by a gas-flame placed underneath. A stream of cold water is made to flow through the outer compartment of the box, the surplus being carried away through the opening upon the front. By means of this apparatus a uniform difference of temperature between the two ends of the middle compartment can be maintained.

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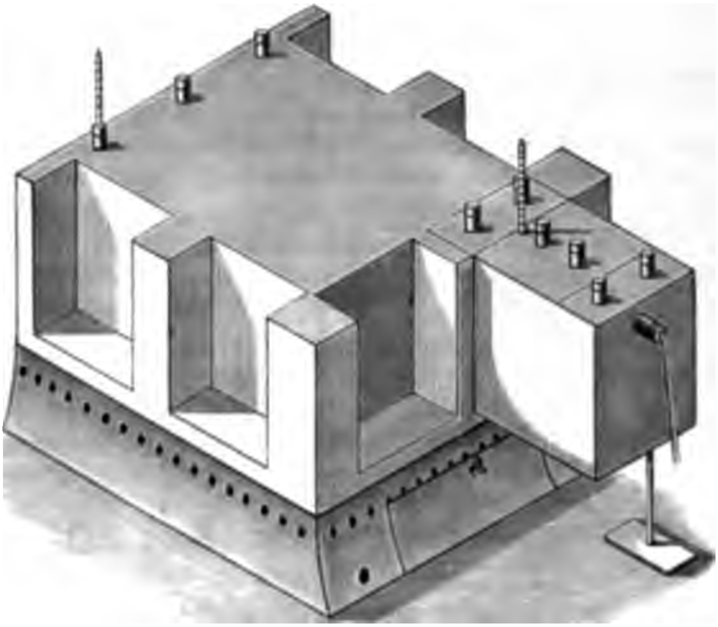
[1] This apparatus, as well as that employed in the experiments on thermotropism, was devised by Professor F. C. Newcombe of this laboratory.

Plasmodium placed in this compartment, with the heated end ranging from 25° to 28° C., and the cold end from 16° to 18° C., could be observed to constantly stream toward the higher temperature[1]. Shutting off the cold water, and placing the plasmodium close to the heated end of the box,



[illegible]

a temperature of 38° C. could be attained. When the temperature reached [illegible] 31° C. the creeping movement became less rapid, but there was no turning from the heat till the temperature rose to between 33 and 34° C. The turning in each case was sudden and decided, and a rapid creeping movement away from the heat took place. The plasmodium must in all this work be kept well supplied with moisture, else it either dies or assumes the sclerotium form. These facts may be taken as another evidence of the extreme sensitiveness of the organism and its power to protect itself against unfavourable conditions.

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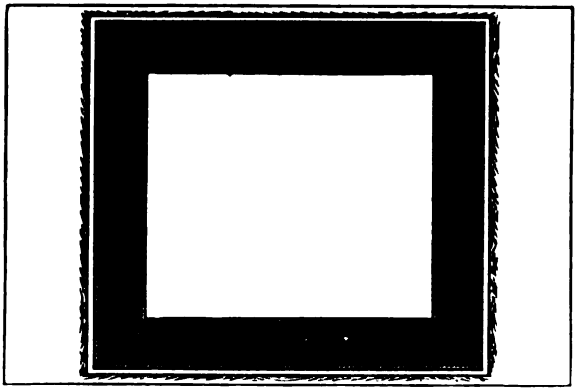
[1] [illegible]

[illegible]

Raising the temperature by means of an additional flame applied directly under the box used, to about 48° C., was followed by a cessation of all apparent movement; and upon raising it still higher, it was found that the plasmodium dies from a very short exposure to a temperature of 52°–53° C.



Woodcut 5.

RESULTS OBTAINED FROM OTHER TEMPERATURE-CHANGES.

After trying various contrivances for microscopic examination of the behaviour of plasmodium, the following was found most satisfactory: a glass slide, 3 in. x 2 in., and a cover-glass, 2 in. x 2 in., were separated by a double layer of blotting-paper one-fourth of an inch wide, as shown in Woodcut 5. In this way a small cage is formed sufficient to contain the necessary moisture, and as the plasmodium is not in the least geotropic, the thin glass with the plasmodium spreading upon it can be kept uppermost, and so brought near the objective. To transfer the plasmodium to the glass, a portion was separated from the mass by cutting the strands across and then loosening it from the substratum with a soft camel's-hair brush. In order that it may have time to recover from the shock of the transfer, and become well spread out, it is best to do this the day before it is needed for use.

One of these cages placed upon the stage of a microscope contained in an apparatus similar to Sachs' warm-chamber[1], gives excellent opportunity for observing the plasmodium and noting any changes in currents or position.

The space surrounding the well containing the microscope was packed with snow and salt, and a temperature of from −2° to −3° C. could thus be easily attained. Down to about 1° C. there was no change from lowering the temperature, either in position or rapidity of the currents. But from this point to about −1° C. there was a gradual slowing of the currents, and between −2° and −3° all movement stopped. If the exposure to this latter cold were but for a few minutes, the currents would soon start if the temperature were raised to 1–2° C.; but if the exposures were made for an hour, in no case was it possible to revive them. So it appears that while a temperature of from −2° to −3° C. does not instantly kill this plasmodium, it will do so if the plasmodium is exposed to it for any length of time.

SUMMARY.

Concerning rheotropism it is found that up to a certain point the plasmodium is positively rheotropic, but that a very slight increase in the strength of the current causes it to become negatively so, and that any considerable increase in the strength of the current causes it to move entirely away from the water. But the question of why the organism has taken on the habit of positive rheotropism is regarded as unanswered; Stahl's explanation, to the effect that it has found this a convenient way of reaching the surface when aroused by warmth and moisture, seeming inadequate to account for the facts, and no other than this has as yet been proposed.

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[1] [illegible]

Concerning thermotropism, it is found that the plasmodium will live in an atmosphere ranging from –2° to 52°–53° C., and that it remains positively thermotropic up to 33°–34° C., but becomes negatively so above that point. In both sets of experiments we find additional evidence of the universally observed, but unexplained, sensitiveness of naked protoplasm, coupled with a remarkable power to protect itself when placed in unfavourable conditions.

The Formation of the Sexual Nuclei in Lilium Martagon:

II. Spermatogenesis.

BY

ETHEL SARGANT.

With Plates X and XI.

IN the first part of this paper I described the three nuclear divisions which take place within the embryo-sac of *Lilium Martagon* and form the nucleus of the ovum there. The reasons which led me to undertake a fresh examination of material rendered classical by previous research will be found at the beginning of that part. My object was to examine each of those three nuclear generations with the single aim of determining whether there was ground for the belief that any one of the nuclei in that series was formed of chromosomes derived from those of the previous generation by transverse fission. Dr. Haecker's hypothesis, which I have discussed elsewhere (I, pp. 445-9[1]), demands that one of the three nuclei in question should be so formed[2]. It further demands that in the parallel series of four nuclear generations which terminates in the formation of the male pronucleus

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[1] I: [illegible] refer [illegible] Part I of this paper, Annals of Botany, [illegible], 1897.

[2] Dr. V. Haecker: [illegible] of the Chromosomes in the Sexual Cells as [illegible], Annals of Botany, ix, 1895.

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within the pollen-tube a similar transverse fission of chromosomes should occur.

My observations on the oögenesis of *Lilium Martagon* give no support to this view. They confirm the conclusion of all previous observers in showing that the chromosomes of each generation are formed by longitudinal fission from the parent chromosomes. I have now concluded a corresponding set of observations on the spermatogenesis of the same plant. Here again we have a series of nuclear generations to be examined so closely that, if a transverse fission of chromosomes should really take place in any one of them, the signs of it may not escape observation.

So minute an inquiry into the details of karyokinesis as that required for this purpose cannot fail to furnish evidence on such general questions as the relation between nucleus and cytoplasm, the function of the nucleolus, and so forth. But one general result obtained from comparison of the spermatogenetic with the oögenetic series requires special mention. The first karyokinesis of either series differs in so many details from those which succeed it, that it may be said to present a different type of nuclear division. Professor Farmer has already pointed out the difference between the first and second nuclear divisions in the pollen-mother-cell of *Lilium*, applying to them the zoological terms heterotype and homotype[1]. How far this nomenclature is justified from a zoological standpoint, I am not competent to determine. But, using the words strictly in the sense suggested by their etymology, they seem to me peculiarly applicable to the case of *Lilium Martagon*. The second and third divisions of the micropylar nuclei in the embryo-sac of that plant follow the vegetative type of karyokinesis (I, p. 468). This is quite evident when Figs. 30 and 42 in Part I are compared with Fig. 6. The nuclear divisions in question differ from a vegetative division in the number of chromosomes, and in no other respect. They may properly be called homotype. The same term is equally applicable to the three later divisions of the spermatogenetic series.

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[1] J. B. Farmer, Ueber Kerntheilung in Lilium-Antheren, &c., Flora, 1895, Heft 1. See also Farmer and Moore, On the essential Similarities existing between the heterotype nuclear Divisions in Animals and Plants, Anatomischer Anzeiger, xi, 1895, Heft 3.

The first division of the pollen-mother-cell nucleus in *Lilium Martagon* is similar in every detail to that of the primary embryo-sac nucleus. Both divisions exhibit a type of karyokinesis differing very widely from that of vegetative nuclei. The difference is sufficiently great to justify the use of the term heterotype.

In a communication at a recent meeting of the British Association (Liverpool, 1896), I attempted to define the difference between these forms of karyokinesis in the particular case of *Lilium Martagon*[1]. My observations on its spermatogenesis were not then completed, and only three out of the four nuclear divisions from that series were described. Now that the details of all the critical nuclear divisions on either side have been published, we have the materials for a detailed comparison of the two types in this one instance. Such a comparison may be of value as a basis for future generalisation, for the terms heterotype and homotype have only lately been adopted by botanists. They have a definite meaning, and it is time that their use should be sanctioned if the zoological analogy can be maintained, or that equivalent terms should be found if such analogy is misleading. The first step to this end is that the botanical meaning of the words should be clearly defined.

Little need be said as to the fixing and staining methods which have been applied to the anther. In the main they resemble those which were found suitable to the ovule (I. p. 47, and Appendix). I have maintained the practice of arranging the preparations in two parallel series, one prepared from material fixed in absolute alcohol, the other from material fixed in one of the osmic acid mixtures. As the tissues of the anther are more easily penetrated than those of the ovule, it has been possible to make greater use of aqueous fixing-solutions. Details of these processes are given in the Appendix at the end.

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[1] Report of the British Association, 1896, p. 1021.

Hand-sections have proved of great value for purposes of comparison with thin serial sections in the study of the first division of the pollen-mother-cell nucleus, but they have not been used to illustrate the two following divisions. The history of the fourth nuclear division in this series—that which takes place in the pollen-tube—has been worked out almost entirely by means of hand-sections from alcohol-material. This is the only part of the work in which a single fixing method has been used to the exclusion of any other.

FORMATION OF THE NUCLEUS OF THE POLLEN-GRAIN.

The anther of *Lilium Martagon* differs from the ovule in possessing a definite archesporial tissue. This grows rapidly by repeated cell-division from the time of its first formation until the pollen-mother-cells are formed. Cell-division then ceases in the loculi for some time, during which the nuclei of the pollen-mother-cells pass through a period of growth and development which corresponds in every detail to that following on the differentiation of the embryo-sac nucleus.

The nuclear divisions in the archesporial tissue are precisely similar to the vegetative divisions described in the first part of this paper (I, p. 451). The chromosomes are somewhat crowded on a small spindle, and it is therefore difficult to find examples in which they can be counted with certainty, but there are always about twenty-four. We may conclude that when the pollen-mother-cell is differentiated, its nucleus is built up of twenty-four chromosomes.

In an ordinary flower-head of *Lilium Martagon*, in which ten or twelve buds are still sessile and crowded together at the top of the flower-stalk, anthers from the youngest buds will usually show archesporial tissue in which there is no lack of nuclear divisions. I possess a series of preparations from ten buds of such an inflorescence. The whole flower-head originally consisted of twelve buds, two of which were accidentally destroyed during the process of preparation. The lowest buds in the head were shortly stalked, and stood somewhat apart from the others. The outer perianth-leaves of each bud were broken off, and the rest of it was cut into a series of transverse sections in which all the parts are shown in their proper position. Archesporial nuclear divisions are frequent in the anthers of the two youngest buds. In the third the pollen-mother-cells are differentiated, and the first indication of an embryo-sac can be seen in the median ovules (I. p. 449). Sections from the lower buds show every stage in the history of the pollen-mother-cell nucleus, from its first formation to the differentiation of the spirem-ribbon within it.

Such a series of preparations serves two purposes. It may be considered as a sort of index to the nuclear stages included within it, giving direct evidence as to their seriation; and if we have any means of determining the difference of age between buds at the beginning and those at the end of the series, we know at once about how long the nuclei of any particular tissue in those buds have taken to pass from one stage to another. I should suppose that such a bud as the third in our series would take a week or ten days to attain the size of the lowest bud, and this must also be the time taken by the pollen-mother-cell nuclei to develop from the resting to the spirem-stage.

The whole interval between the complete differentiation of the pollen-mother-cell and the formation of the young pollen-grains within it may conveniently be divided into four periods.

1. The nucleus of the pollen-mother-cell grows larger and alters in structure, finally assuming the well-known spirem-condition (Figs. 1–3 a).

2. Twelve chromosomes are formed from the spirem-ribbon, and lie loose in the nuclear cavity (Figs. 4–10).

3. The first karyokinesis of the pollen-mother-cell nucleus separates the two halves of each chromosome. Cell division follows this nuclear division (Figs. 11 a–15).

4. The second karyokinesis divides the nucleus of each daughter-cell into two, and is followed by a corresponding cell-division (Figs. 16–22).

Measurements of the pollen-mother-cell nuclei in the earliest stage show that they are then about the same size as the embryo-sac nucleus when just formed. They remain, however, for a much shorter time in the resting-state, and during that period do not increase so much in size. The embryo-sac nucleus is in the resting-state for about a fortnight, and during that time its mean diameter is nearly doubled (I, p. 455, and Figs. 10, 11). The nuclei of the pollen-mother-cells, on the other hand, do not retain the structure of a resting-nucleus for more than a day or two, and their mean diameter increases during that time by less than half its length. Indeed, the pollen-mother-cell nucleus is throughout its development smaller than the embryo-sac nucleus at corresponding stages. No doubt this is connected with the small size of the pollen-mother-cell as compared with the embryo-sac. I have drawn the pollen-mother-cells of the figures numbered 1, 2, 3–15 on the same scale as Figs. 10–26 in Part I for purposes of comparison. When this scale has proved too small to show the necessary detail, portions of nuclei at corresponding stages have been drawn under a higher magnification (Figs. 1 *a*, 2 *a*, &c.).

The structure of the resting pollen-mother-cell nucleus corresponds exactly to that of the resting embryo-sac nucleus, though the detail is not quite so clear. A network of threads which seem to anastomose is partly obscured by the cloudy substance staining like dilute chromatin, which I have called amorphous chromatin. Here and there, in very deeply stained preparations, it can be seen that the threads are dotted (× in Fig. 1 *a*), but this is less clear than in the embryo-sac nucleus. There are usually several spherical nucleoli (Fig. 1).

As the nucleus increases in size, the amorphous chromatin is seen to be less generally distributed over the nuclear section. It is aggregated round the threads of the chromatic network, and seems to have diminished in bulk. Some considerable quantity of amorphous chromatin is still present when the nuclei have completed their growth in size, and even when indications begin to appear of the approach of synapsis.

The passage from the resting-state to the contracted condition known as synapsis is very instructive in these nuclei. No independent evidence of the seriation of stages could be obtained from preparations of the corresponding period in the embryo-sac. The comparative ages of the nuclei, where the difference was slight, were settled on internal evidence only. But in a single longitudinal section from a young anther, the pollen-mother-cell nuclei at one end may be in the late resting-condition, while those at the other are completely contracted. The intermediate nuclei then exhibit an orderly series of transitional forms.

The approach of synapsis is first indicated by the appearance of drops of nucleolar matter adhering to the chromatic network. The larger of these drops can be identified by their reddish colour in sections from alcohol-material stained with methyl-green and acid fuchsin. The nucleoli are still spherical and well defined, but often vacuolated. A little later the nucleoli lose their well-defined outline, the nuclear membrane becomes indistinct, and the chromatic threads show a tendency to collect round the nucleoli at one side of the nuclear cavity (Fig. 2). This contraction becomes more and more pronounced, the nucleoli collect into a shapeless mass of ill-marked outline, and the nuclear membrane seems to have disappeared as a continuous structure. Its place is taken by a sort of web of cytoplasmic fibrils. In sections stained with methyl green and acid fuchsin it is to be remarked that the nucleolar mass is often coloured green in the thicker parts (as, for example, near *x* in Fig. 2). Possibly it has dissolved the amorphous chromatin which was still visible in the later stages of the resting-nucleus. The edges of this greenish mass are always of a washy red colour and the nuclear sap in its neighbourhood is coloured with red.

Even in such a preparation as that from which Fig. 2 is drawn, it can be seen that the chromatic threads have grown in thickness. Sections from material fixed in Flemming's solution show the structure of the thread in detail. It is irregularly thickened: here and there drops of nucleolar matter adhere to it (*n*, *n*, in Fig. 2 *a*). In the broader bits of thread from very clearly stained preparations, a double row of dots can sometimes be made out.

The nucleus of the pollen-mother-cell has now entered on the period of contraction called synapsis, which precedes the formation of the spirem-thread. It is a condition which persists for several days, and the phenomena which distinguish it are familiar to all who have worked at the subject; but it has been considered by most observers to be an artificial state produced by the action of reagents. This is not the place in which to enter into an account of the controversy which has arisen on various points connected with this subject. I shall simply describe my own observations on this period in the history of the pollen-mother-cell in the single case of *Lilium Martagon*. A fuller account of the evidence will be given than would be necessary if no controversy existed.

The appearance of the nucleus in the contracted state depends very much upon the thickness of the section examined. If it is thick enough to include the whole depth of most of the nuclei contained in it, the general appearance of each is that shown in Fig. 3, which is drawn from a hand-section of alcohol-material. All that is visible of each nucleus in such a section is a swollen nuclear cavity containing a ball of chromatic substance, which adheres to one side of it. The ball is clearly formed of close coils; some of them can be distinguished from each other near the edge of the ball. No definite nuclear membrane can be made out; but this is not conclusive in such a preparation, for its thickness necessarily renders details indistinct.

Before proceeding to a description of thin serial sections, which give details of the nuclear structure in this stage with great precision, I will give a complete account of those observations on living material which have been mentioned already (I, p. 451). It is clear that such observations are of importance, for if well established they will settle the question as to the natural or artificial character of the contraction we are dealing with.

It has already been said that the nuclei of the pollen-mother-cells remain in the contracted condition for several days. It is therefore easy to find material of the right age. Anthers are chosen from buds which stand midway between the highest and lowest buds of such an inflorescence as that described on page 190. The only difficulty in their preparation lies in cutting sufficiently thin sections from fresh material. This can generally be done in elder-pith with a dry razor. The sections were mounted in sap expressed from the perianth-leaves of the bud, and were covered with a glass slip. They were examined with a homogeneous immersion objective of 2 mm. focal length, N.A. 1.30, from Zeiss' apochromatic series; eye-pieces 4 and 6 were used. The Powell and Lealand condenser was lowered until the angle of the incident light—from an incandescent gas-burner—was sufficiently narrow to give a clear outline to the unstained nucleus[1].

Notes were taken of the observations made in this way on three separate occasions, and outline drawings of the pollen-mother-cell and its nucleus were kept. Within the rather obscure wall of the pollen-mother-cell was seen a clear space, well outlined against the granular protoplasm filling the rest of the cell. One side of this space was occupied by a rather opaque spherical ball.

It now became necessary to identify the features of this outline. On two mornings the section was roughly fixed and stained by Strasburger's well-known method of allowing methyl green dissolved in one per cent. acetic acid to run in under the cover-slip. This very imperfect fixing method produced deformation in the shape of the clear space, but the spherical ball stained an intense green, and thus showed the presence of chromatin. The section cut on the third morning was particularly successful, and was preserved for future reference by laying it in much diluted Merkel's solution for half an hour, and then staining with Mayer's haemalum. I could not detect any alteration in the shape of the nuclei after this fixing and staining process. The fixed and stained section, though thin for one cut from fresh material, cannot be compared with a good hand-section from a hardened anther, and the structure of the nuclei is further obscured by the heavy stain; but there can be no doubt that the nuclei are really in the condition of synapsis. Their outlines correspond perfectly well with that drawn from a nucleus in the fresh section before fixing.

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[1] This is the arrangement of optical conditions which I have found to give the best results. When the aperture of the incident light was increased by the use of an iris diaphragm, the definition of the image was less perfect than that obtained by [illegible]

We may now proceed to examine the structure of the contracted nucleus in detail by means of sections considerably thinner than its mean diameter. The appearance of any particular nucleus in such a section will depend greatly upon how the contracted part lies with reference to the plane of the section. If the knife has passed through the area over which the chromatic ball is in contact with the boundary of the nuclear cavity, then the section of the nucleus will have an outline resembling that of Fig. 3*a*. But if the section is roughly parallel to the area of contact, the chromatic ball may appear as a circular disk within the nuclear cavity, but quite free from it. In this case the whole section of the chromatic ball commonly drops out, leaving the nuclear cavity as an empty space of irregular outline. It often happens also that the sections are injured by the knife, perhaps because the absence of a definite nuclear membrane weakens the structure of the whole nucleus. At any rate, fragments of the chromatic thread and bits of nucleolar matter are constantly found outside the nuclear cavity, and the whole preparation is apt to look ragged and untidy.

Neglecting such details as may be fairly attributed to imperfect preservation, we find that there are three features characteristic of synapsis—a more or less complete disappearance of the nuclear membrane, partial solution of the nucleolus, and contraction of the chromatic thread. The structure which we have hitherto called the chromatic ball is seen to consist of the chromatic threads closely coiled round a mass of semi-fluid matter, which must be chiefly of nucleolar origin. As we have seen, the colouring in preparations stained with methyl-green and acid fuchsin suggests that there is also some amount of chromatin dissolved in this mass. The chromatic threads in nuclei which have just entered on synapsis—for instance, those at one end of a loculus which shows resting-nuclei at the other end—resemble that drawn in Fig. 2*a*. Such threads are irregularly thickened, and the double row of dots can only be made out here and there. The cytoplasm which surrounds the nucleus often shows fibrillar structure at this time, and in the absence of a proper membrane the nuclear cavity is outlined by a denser web of cytoplasmic fibrils (Fig. 3).

The structure I have just described is characteristic of the earlier period of synapsis. Later on—in such a nucleus, for example, as that drawn in Fig. 3*a*—the nuclear membrane begins to reappear, nucleoli are slowly reconstructed out of the amorphous nucleolar mass, and the coils of the chromatic thread become looser. The thread itself is now of uniform breadth, and is bordered by two regular rows of dots (Fig. 3*b*). These changes proceed slowly until the chromatic coils occupy nearly the whole of the nuclear cavity, which is once more bounded by a well-marked membrane. The nucleoli are more or less spherical and of definite outline. No trace of an amorphous mass remains. The spirem stage is reached when the chromatic coils, which can now be seen to form a continuous ribbon, occupy the whole of the nuclear cavity.

The structure of the pollen-mother-cell nucleus in the spirem-condition is perfectly clear. It is a good deal smaller than the embryo-sac nucleus at the corresponding stage (cf. Fig. 4 with Fig. 14 in Part I), and the coils of ribbon are more closely packed in the nuclear cavity. The ribbon itself looks broader than the ribbon of the embryo-sac nucleus; but this is the effect of unconscious comparison between the breadth of the ribbon and the size of the nucleus. Wherever the full breadth of the twisted ribbon can be seen, it turns out to be about the same in both. Sometimes the nucleus has a single nucleolus at this stage (Fig. 4), but commonly there are two or more. The differentiation of the chromatin-granules on the ribbon is perfectly clear even in thick sections from alcohol-material (Fig. 4); thin tangential sections from nuclei fixed in Hermann's solution show it very beautifully, as in the preparation from which Fig. 4*a* is drawn. This preparation is stained with Flemming's triple stain (I, p. 474 Appendix), in which the characteristic colour of chromatin is a dull purple, and that of nucleolus and cytoplasm orange-red. The matrix of the spirem-ribbon stains red under this treatment, while the granules which border it are dark. Other double stains bring out the same distinction: the ribbon itself stains like the nucleolus and cytoplasm, while the dots take the colour of chromatin.

We have now traced the history of the pollen-mother-cell nucleus through the first period of its development. That period is occupied by the formation of the spirem-ribbon. We have seen that it is differentiated while the nucleus is in the contracted condition known as synapsis, and it seems probable that the linin-matrix of the ribbon is formed in great part from the substance of the half-dissolved nucleolus. The double row of chromatin-granules can be first demonstrated with certainty during the condition of synapsis, but there is reason to think that it was formed by the fission of the pre-existing single row before the final contraction took place. In all these points, as also in minor details, the development of the pollen-mother-cell nucleus agrees exactly with that of the embryo-sac nucleus.

The nucleus of the pollen-mother-cell remains for a short time in the spirem-condition. When development proceeds again, it still shows the closest agreement with that of the embryo-sac nucleus. As before, however, the smaller size of the former nuclei renders detail less clear. On the other hand, we again have evidence as to the seriation of the developmental stages from the position of the mother-cells in the loculus. Moreover, the smaller diameter of the nuclei is of advantage in one way; for though an untouched nucleus is only to be met with in hand-sections, yet serial sections of 15 μ thickness include more than half the depth of favourably placed nuclei, and such preparations form a valuable link between those which show the complete nucleus, in which details are obscured by the thickness of the section, and the clear but fragmentary preparations of nuclei found in serial sections of thickness varying from 5 μ to 10 μ.

As in the embryo-sac nucleus, longitudinal fission is found in parts of the spirem-ribbon before it has been divided into lengths by transverse division (× and ×′, Fig. 5)[1]. Articulation or beading of the spirem-ribbon precedes the formation of these loops. Short segments of the ribbon, including two or more pairs of granules, become slightly swollen, and the ribbon between such segments is rather narrower than in other places (Fig. 4*a*). In the preparation from which Fig. 5 is drawn, indications are already seen of the approaching solution of the nucleolus: the nuclear sap is coloured cloudily in places. Thus at × the space between the two rows of granules is slightly coloured as compared with the surrounding space, but this only occurs in the neighbourhood of the nucleolus. At ×, for example, the space within the loop is quite clear. Details of the process of longitudinal fission are seen in such tangential sections of nuclei as that shown in Fig. 5*a*, which is from the same anther as the nucleus drawn in Fig. 4*a*.

The separation of the two rows of granules is nearly if not quite complete when the spirem-ribbon first shows indications of a division into lengths. Fig. 6, for example, is drawn from a nucleus near that from which Fig. 5 is taken; at × a transverse division has just taken place, and when the section is narrowly examined, longitudinal fission can be made out in the ribbon wherever the nuclear sap is uncoloured. Solution of the nucleolus (*n*) has clearly begun, and the

[1] J. B. Farmer, Journal of the Royal Microscopical Soc., Oct. 1893, p. [illegible]

coloured nuclear sap seems to run in between the two rows of granules like a soap-film between knitting needles. Thus the space between such rows is often more highly coloured than the surrounding region, as may be seen to the right of Fig. 6. In that part of the nuclear section it almost seems as if fission of the ribbon had not taken place, or was incomplete; but on the left of the figure the sap is clear, and no mistake is possible.

Division of the spirem-ribbon has now taken place in two directions. The rows of chromatin-granules which border it have been separated from each other by longitudinal fission, and the double thread thus formed has been transversely divided into lengths. These lengths, each consisting of two dotted filaments much twisted on each other, are in fact immature chromosomes, and when they have assumed a more compact shape, we shall find that there are twelve of them.

The development of the mature chromosome from each pair of twisted filaments begins at once, and it is a curious fact that the process is accompanied by all the signs of synapsis. I have already spoken of the gradual solution of the nucleolus. In Figs. 7 and 7 *a* we see an amorphous mass of nucleolar matter which recalls an earlier stage (cf. Fig. 3*a*). The twisted threads cluster round this mass at one side of the nuclear cavity, and the nuclear membrane becomes obscure or actually disappears. The contraction itself is clearer at a rather later stage than that shown in Fig. 7 *a*, and is never so well marked as in the embryo-sac nucleus (I, Fig. 18), but its character cannot be mistaken. In examining anther-sections under a low power, I have frequently been unable to decide whether the contracted nuclei at one end of the loculus were younger or older than those in the spirem-stage at the other end. Indeed, it requires fairly high magnification, and some attention, to distinguish between stages of synapsis which are just passing into the spirem-condition and the contracted stages which follow it.

It should be noticed that in the stage represented in Fig. 7 *a*, the presence of coloured sap between the dotted filaments is very conspicuous. As the nucleus emerges from the contracted state and the nucleoli are reconstituted, this feature disappears. The filaments, which looked ragged when first formed—an appearance easily understood when we remember the lumpy distribution of the linin in the spirem-ribbon during longitudinal fission (Figs. 4*a* and 5*a*)– become of uniform thickness during this second synapsis (Fig. 8), and before the end of that period two rows of dots can be made out in each filament. The young chromosome now consists of two lengths of ribbon twisted round each other[1]. Each length bears two rows of dots (Fig. 8*a*). These four rows of dots are the product of two successive longitudinal fissions. The ribbons continue to grow in breadth (Fig. 9*a*), while the nuclear membrane reappears and the nucleolus resumes its definite outline (Fig. 9). No further change in the structure of the chromosome takes place for some time. It continues to grow shorter and broader, and the twist of its two segments on each other becomes closer.

The chromosomes which will take part in the first karyokinesis of the pollen-mother-cell nucleus are now nearly mature. They lie loose within the nuclear membrane in company with the nucleoli (Fig. 10). Each consists of two segments which are already perfectly distinct, and which will be separated from each other in the course of the coming karyokinesis. The chromosomes of a vegetative nucleus show no fission until it is mature and has taken its place on the spindle (I, p. 434 and Fig. 5). But in the pollen-mother-cell nucleus we have seen that the fission of chromatin-granules took place within the immature spirem-ribbon many days before karyokinesis. The fission thus begun was completed by the splitting of the spirem-ribbon before it

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[1] [illegible] are [illegible] with each other [illegible] at a later stage [illegible] same [illegible] of [illegible]

had been divided into chromosomes. Thus each chromosome has been divided into two segments from the very outset of its independent existence, and these segments were formed by a process of longitudinal fission.

It is a remarkable fact that in such an immature chromosome as that drawn in Fig. 9 *a*, each segment exhibits a fission of chromatin-granules exactly resembling that which preceded the complete fission of the whole chromosome. We are naturally led to expect that this also will be completed at a later stage of development, and that such a chromosome as we have described has an essentially fourfold structure. This view is the more tempting when we recall the formation of tetrad-groups, which is so characteristic of the animal spermatocyte. Dr. Brauer has shown that in the case of *Ascaris* each tetrad-group is the result of a double longitudinal fission [1].

The chromosomes of the pollen-mother-cell nucleus of *Lilium Martagon*, however, undergo a change, on the final disappearance of the nuclear membrane, which effectually conceals all traces of the incomplete fission just described. Up to the time of that disappearance the segments of each chromosome show the differentiated structure drawn in Fig. 9 *a*. But as the membrane vanishes, the colouring of the chromosome-segments becomes uniform. Each is apparently homogeneous. There is no contrast between cyanophilous granules and erythrophilous ribbon, but the whole chromosome stains uniformly like chromatin (Fig. 11). A similar change has been observed at the corresponding period in the history of the embryo-sac nucleus (I, p. 461).

The true structure of the mature chromosome is very much obscured by this change in colouring power. However tightly its segments were twisted on each other, they could still be distinguished without difficulty so long as each consisted of a ribbon outlined with a dark border of dots. Now, however, the segments are indistinguishable where they cross each other. The loops between them are sometimes fairly large,

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[1] Brauer, Zur Kenntniss der Spermatogenese von Ascaris megalocephala, Arch. f. mikr. Anat., V. 42, 1893.

and in such cases the twist of the segments on each other can be made out (Figs. 11 and 11 *a*). When the twist is tight, the loops may appear as circular dots in the otherwise seemingly solid chromosome, or no loops at all may be visible. This is particularly apt to occur in preparations from material fixed with absolute alcohol. The real structure may be demonstrated in such cases—at the cost of the preparation—by first treating the nucleus with warm 50% acetic acid to clear the structure[1], and then allowing fuming hydrochloric acid to run in under the cover-slip[2]. The cellulose and cytoplasmic structures have all been more or less dissolved by the acetic acid. The chromosomes stand out, their structure unaltered, but with a peculiar highly refractive appearance. It is well to choose a particular chromosome for observation under a high power before running in the fuming hydrochloric acid. The action of this reagent is very sudden. The chromosome retains its solid homogeneous appearance for some time, but then swells up, and for a few moments shows two spongy segments, each clearly outlined by a broken black line. The twist of these segments on each other is quite clear, but the whole chromosome disappears very quickly. Chromosomes fixed in Hermann's solution cannot be dissolved in this way.

The disappearance of the nuclear membrane and the formation of the spindle can often be beautifully traced in the mother-cells of a single loculus. Fragments of nucleolar matter can sometimes be seen among the chromosomes after the nuclear membrane has gone. At the same time a number of large erythrophilous granules appear in the cytoplasm (Fig. 10 *a*). These are doubtless of nucleolar origin

The chromosomes seem driven together in a heap on the disappearance of the boundary between nucleus and cytoplasm (Fig. 10 *a*). The spindle-fibres soon make their appearance at two or three points in the cytoplasm (Fig. 10 *a*), whence they extend towards the chromosomes. The spindle thus formed is rarely symmetrical at first (Figs. 10*a*, 11, 11*a*)[1]. The chromosomes soon begin to scatter, being attached to bundles of fibres (Fig. 11 *a*).

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[1] E. Zacharias, Ueber das Verhalten des Zellkerns in wachsenden Zellen, Flora, 1895, Ergänzungsband, p. 241; quoted in Zimmermann's Morphologie und Physiologie des pflanzlichen Zellkernes, 1896, p. 27.

[2] Strasburger, Ueber Kern- und Zelltheilung, 1888, p. 146.

As the spindle becomes symmetrical, the chromosomes are arranged in an equatorial plate. Two very well-marked bundles of spindle-fibres are then attached to each chromosome (Fig. 12)[2]. One of these bundles is fixed to each segment, and they point to opposite poles. The two segments move apart from each other as if each were pulled to its pole by the fibres attached to it. During the process of separation the whole chromosome becomes much contorted. This is not surprising when we consider how tightly the segments of each chromosome are usually twisted on each other, and that they must untwist as they are pulled apart. Adjacent chromosomes may assume very different shapes (Fig. 12). This depends very much on the point of attachment of the spindle-fibres, which may be in the middle of the chromosome or near one end. The knotted and strained appearance of the segments just before complete separation is much less conspicuous here than in the first division of the embryo-sac nucleus (I, p. 462). Compare for instance Fig. 13 in this paper with Fig. 23 in Part I. It seems as if the strength of the pull exercised on the chromosomes by the spindle-fibres varied to some extent with the size of the cell.

The daughter-chromosomes in the diaster-stage are commonly V-shaped, the angle pointing towards the pole which it approaches (Figs. 13, 14). This angle is often bent back (Fig. 14)[3]. When the two groups of daughter-chromosomes reach the poles, they cluster closely together, and the deflexed angle forms part of a depression in the daughter-nucleus—the 'polfeld.' A cell-plate is formed between the two nuclei (Fig. 15), and is shortly followed by a cell-wall.

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[1] W. Belajeff, Zur Kenntniss der Karyokinese bei den Pflanzen, Flora, Ergänzungsband, 1894, p. 438.

[2] W. Belajeff, l. c. p. 439. See particularly Fig. 15 from *L. candidum*.

[3] See Postscript.

The number of the chromosomes taking part in the first karyokinesis of the pollen-mother-cell nucleus is most easily counted while they form a nuclear plate. A single longitudinal section from an anther of the right age contains hundreds of such figures, but there is some difficulty in exact counting, because the chromosomes are crowded on the spindle. I finally adopted the following method:—Two hand-sections were closely examined for spindles more or less perpendicular to the plane of the section. Before any attempt was made to count the chromosomes, certain tests were applied to each figure. The objective was raised until the whole nuclear plate was indistinct. If no part of the section in its neighbourhood was then in sharp focus, the figure was rejected. The same test was applied with a lowered objective. The object of both was to exclude nuclear plates lying on either surface of the section, for chromosomes lying above or below the others might have been removed from such figures in cutting the section. Nuclear plates which stood these tests, but in which the chromosomes were crowded together on one side, were also rejected. Twenty nuclear plates were chosen in this way.

In 9 cases	there were	certainly	12	chromosomes.
„ 9 cases	„	either	11 or 12	„
„ 1 case	„	„	12 or 13	„
„ 1 case	„	„	10, 11 or 12	

The daughter-nuclei formed by the first division never enter the resting-stage. Each is merely a close knot of chromatic ribbon, without nucleoli but enclosed in a nuclear membrane which disappears in the earlier stages of karyokinesis.

When a daughter-nucleus is about to divide, it begins to swell up, and its chromatic coils become looser. As they separate, a certain symmetry is seen in their arrangement (Fig. 16). The coils now become stretched in the direction of the axis of the future spindle (Fig. 17), but the distortion is very slight compared to that of the corresponding stage in the micropylar daughter-nucleus of the embryo-sac. There the young chromosomes have a knotted and strained appearance, which is absent from those we are considering (cf. Fig.

29 in Part I with Fig. 18 in this part). Another difference must also be mentioned. In the embryo-sac the young chromosomes when just formed from the spirem-ribbon are of different shapes, often hooked at one or both ends (I, Fig. 29). Most of those formed from the daughter-nucleus of the pollen-mother-cell are alike and of a perfectly definite shape, which may be described as a V with the angle bent back (Fig. 18, Nos. 1, 2, 3, 4, 5, and 9). This is the characteristic form of the diaster-segments in the previous division (Fig. 14). A few are irregularly twisted (Fig. 18, Nos. 6 and 7), and others still show the median bend characteristic of the V-shape, though they are straightening out (Fig. 18, No. 8). This peculiarity of shape suggests that the daughter-chromosomes of the first division have retained their identity within the daughter-nucleus.

The chromosomes do their best to form an equatorial plate as soon as the spindle is formed. They are necessarily much crowded together. The whole pollen-mother-cell was barely large enough for the spindle of the first karyokinesis. The chromosomes which take part in the second are fully as long as the mother-chromosomes, though less thick. But the pyramidal mother-cell has been divided by a partition into two compartments of awkward shape. The spindle formed in each is usually more or less distorted to fit it (Figs. 20, 22). The long slender chromosomes have to fit as best they may into the equatorial zone of this spindle (Fig. 20).

This crowding together of the chromosomes on the spindle makes observation of the details of karyokinesis somewhat difficult. At least two different accounts have been given of the way in which actual separation of the chromosome-segments takes place[1]. I have found thick sections useless, because in a preparation which includes the whole of a nuclear plate one chromosome cannot be distinguished from another. Very thin sections are apt to be misleading, as they rarely include a complete chromosome. Sections about 7·5 μ thick from Hermann-material are the most useful, and it is well to compare them with others about 5 μ thick. Figs. 16-22 are drawn from such preparations. Of these, Figs. 20 and 22 give the general appearance of the nuclear section as faithfully as I can render it. The others are slightly diagrammatised by the omission of such chromosomes and fragments as would make the figure obscure. In doing this I have of course been influenced in retaining or omitting detail by my own interpretation of the structure. This interpretation is based on very careful study of every detail in the karyokinesis.

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[1] (1) Guignard, Nouvelles Études sur la Fécondation, p. 176; J. B. Farmer, Ueber Kerntheilung in Lilium-Antheren, Flora, 1895, p. 67. (2) Strasburger, Botanisches Practicum, III. Aufl. p. 611.

Most of the chromosomes retain their characteristic shape throughout the process of division (Figs. 19 and 21). The angle of the V lies in the equatorial plane of the spindle, and the legs usually point outwards or towards one pole or the other (Fig. 19). Occasionally the legs point towards different poles, and then the V-shape is lost, though the chromosome retains traces of its original form (No. 8 in Fig. 18, and the middle chromosome in Fig. 19). One result of this arrangement is, that when the chromosomes of a whole nuclear plate are seen sideways there seem to be more than twelve, for it is easy to mistake each leg of the V for a separate chromosome. The whole of the spindle is not shown in the nucleus to the right of Fig. 20, yet fifteen free ends can be counted on it. Besides the V shaped chromosomes, there are usually two or three in each spindle which are straight or irregularly twisted. One end of such a chromosome commonly lies in the equatorial plane, and longitudinal fission of the segments begins there, as is the case also in vegetative nuclei. The segments of V-shaped chromosomes are also formed by longitudinal fission, but they begin to separate at the angle (Fig. 21). Hence most of the diaster segments are V-shaped, but a few are hooked (Fig. 22). The reconstruction of the daughter-nuclei from the diaster-segments goes on in the usual way, and is shortly followed by the formation of a cell wall between them. Each of the four segments of the mother-cell rounds itself off and begins an independent existence as a young pollen-grain, but for some time the envelope of the pollen-mother-cell is still visible round each group of four.

We have now traced the history of the four pollen-grain nuclei through all the steps of their development from the nucleus of the pollen-mother-cell. We have seen that the chromosomes of this nucleus showed at one period a complete division into two segments and an incomplete division of each segment into two. We have seen the two segments separated from each other during the first karyokinesis of the pollen-mother-cell nucleus. It is natural to inquire whether the subsequent division of these segments in the second karyokinesis does not correspond with the incomplete fission visible in each segment before the first took place. This question, however, I am unable to answer. It is difficult to believe in a sudden and complete change of structure in each chromosome at the time when its colouring becomes uniform (p. 202). But no trace of fission can be seen in the segments after that period. It might be possible to trace it from one karyokinesis to the other by microchemical methods, for there is no intervening period of rest, but the few experiments I have tried gave no conclusive result. Whether the daughter-segments separated during the second karyokinesis are formed *de novo* shortly before separation, or whether they date back to an earlier period, they are equally the result of longitudinal fission.

FORMATION OF THE MALE PRONUCLEUS.

The young pollen-grains are soon set completely free within the loculus of the anther by the disappearance of the mother-cell envelope. No further change takes place in the nucleus of each grain for some time, during which the exine becomes thickened and beautifully sculptured on the outer surface (Figs. 23 and 27). The pollen-grain meanwhile grows considerably in length and assumes a curious shape (Fig. 23 *a* and *b*). The resting-nucleus can hardly find room within it, and is often flattened (Fig. 23 *b*). There is a deep fold in the exine, and the inmost part of it continues unthickened. Thus, when the pollen-grain swells up later on, there is a thin region in the otherwise much thickened exine, which is shaped like a narrow gore in a balloon (Figs. 23, 27, 31).

The nucleus of the pollen-grain divides once before it is mature. It is not always easy to hit on anthers in which this division may be seen. Strasburger's classical method for determining the stage of the pollen-mother-cell nucleus[1] is not applicable here, for the exine of the pollen-grain is already perfectly opaque, and even if the stain should penetrate, the nucleus would not be visible. I could not succeed in making the exine transparent by the use of chloral hydrate or other clearing agents. It is out of the question to cut sections by hand without previous embedding, for the pollen-grains fall out of the loculus at once. Thus the only plan was to embed anthers of different ages and cut test sections from each with the microtome. In this way, after many failures, I succeeded in finding the pollen-grain nuclei in the act of dividing. This occurs at a period when the anther is bright orange, but before it has assumed the characteristic red tint. The filament—hitherto so short that the anther may be called sessile—has just begun to grow rapidly.

The pollen-grains in which nuclei are dividing are not arranged in a series within the loculus. On the contrary, one containing a single resting-nucleus may be found surrounded by others which present every stage of division. Here, as in the embryo-sac, there is no independent evidence by which to determine the seriation of stages. The nuclei are small, and there is some difficulty in obtaining clear differentiation by the ordinary staining methods. The figures which give details (Figs. 25, 27) are all from the same preparation, a series of sections 5 μ thick cut from material fixed in alcoholic Hermann's solution. In one slide of this series the staining has been a success, and all the details in the structure of the chromosomes are quite clear. The cytoplasm is very lightly coloured however, and for details of its structure I have referred to another series, cut from absolute alcohol-material and stained with Renaut's haematoxylic eosin (Figs. 24, 28).

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[1] [illegible] III, No. 3, [illegible]

The daughter-chromosomes separate exactly as in vegetative nuclei. Indeed, this is the most typical of the five homotype divisions. The series of diagrams headed 'homotype' in Plate XI are founded on the vegetative karyokinesis (I, p. 451), but they might have been drawn equally well from the division we are now considering. The longitudinal fission of the chromosomes is perfectly clear (Fig. 27).

Both the resting and the dividing nucleus are invariably found close to one wall of the pollen-grain. The spindle is not unusually short. Its long axis is at right angles to the cell-wall near which it lies (Fig. 28). But during the dispirem-period the connecting threads look as if they were crushed between the two daughter-nuclei. The inner one is now much nearer than before to the outer, which is still close to the cell-wall.

I have never succeeded in seeing centrosomes, but a differentiated mass of cytoplasm is very conspicuous both at the inner pole of the spindle (Fig. 28) and on the inner side of the spirem-nucleus (Fig. 24). Radiations can often be traced from this mass into the surrounding cytoplasm. The latter shows a honeycomb-structure very clearly (Figs. 24, 28), but this may possibly be due to imperfect fixing. The exine offers considerable resistance to the penetration of fixing agents.

The two daughter-nuclei continue to lie very close to each other for some time after the connecting threads have disappeared. A difference in size very soon becomes apparent. The generative nucleus is the smaller, and in the mature pollen-grain it is enclosed within a lens-shaped naked cell (see M. Guignard's beautiful figure: Nouvelles Études, Plate X, Fig. 27). During the interval between the formation of two daughter-nuclei and the dehiscence of the anther, each pollen-grain swells so that the outline of its equatorial section is circular, and it also grows larger (cf. Fig. 30 with Fig. 28). No change takes place in the structure of the two resting-nuclei until the pollen-grain reaches the stigma where it will germinate.

I have often watched hive-bees visiting plants of *Lilium Martagon* in search of honey. The flower when it first opens hangs downwards, the petals are quite flat, and the stigma is seen below them like the clapper of a bell. It is considerably longer than the stamens. Later on in the same day the petals curl up, leaving the circle of anthers exposed round the long pendulous stigma. The three older anthers usually dehisce about this time. Next morning all the anthers have dehisced, but the stigma still hangs downwards. A bee in search of honey climbs in by the petals and clings to the stamens while it is sucking. Thus the under side of its body gets well dusted with pollen from the versatile introrse anthers, but the stigma is untouched. On the third morning, however, when the anthers have shed most of their pollen, the style bends upwards, and forms a convenient perch on which the honey-seeking bee may alight. In this way it is sure to be touched with pollen from the lower part of the bee's body. Bees invariably begin with the lowest flower of an inflorescence and work upwards. Cross-fertilization is ensured, as in the Foxglove, by the proterandry of the flowers and their centripetal arrangement on the stalk.

The stigma of *Lilium Martagon* is three-lobed, and each lobe is outlined by a depression in the stigmatic surface. It is also bisected for a part of its length by a cleft, the three clefts meeting in the centre of the stigma and running down into the channel of the style (Fig. 29). On either side of each cleft is a slight ridge, covered, like the rest of the stigmatic surface, with stigmatic hairs. More pollen is commonly deposited on the ridges bordering the two upper clefts in the upturned stigma than on the ridges of the third.

It is a laborious business at best to hunt for dividing generative nuclei in pollen tubes creeping among stigmatic hairs. I have identified such stages most easily in hand-sections from alcohol-material. The stigma is cut from the style about a millimetre below the stigmatic surface, and the thick slice thus obtained is stained *en bloc* with a dilute solution of Mayer's haemalum in 1 % potash alum. It is dehydrated in the usual way, cleared in clove-oil, and examined in xylol under a simple microscope. The lobes are separated, and those only are used which are well covered with pollen-grains. Sections are cut between slices of pith in a direction perpendicular to the cleft in the lobe, razor and material being kept wet with xylol. The sections are mounted at once in Canada-balsam, and examined with an immersion objective. It is easy to find pollen-tubes and germinating pollen-grains containing the generative nucleus in the spirem-condition without nucleolus or nuclear membrane, but later stages are rare. Some stigmas which had been cut and fixed between half-past one and two o'clock on a sunny day gave me the best results.

Very soon after the pollen-grain reaches the stigma, the spirem-ribbon is differentiated in the generative nucleus. The vegetative nucleus continues in the resting-state (Fig. 30). The pollen-tube grows out at the place where, as we have already seen, there is a thin-walled stripe or gore in the exine (Figs. 30 and 30 *a*). It pushes downwards among the stigmatic hairs, on the top of which the pollen-grain rests. The tube twists over and round these hairs until it reaches their base and crawls between them. When the pollen-grain is thus firmly anchored to the stigma, its nuclei enter the tube, the vegetative nucleus usually going first[1]. Sometimes the upper end of the pollen-tube remains within the exine until the cell-contents have travelled away from both. In other cases the exine may fall off (as in Fig. 31), and the turgid pollen-tube stand nearly upright on the stigma (*p. t.* Fig. 31). The division of the generative nucleus is most easily observed when it takes place within such an up-standing tube (Figs. 32, 33), or when, as sometimes happens, the pollen-grain has fallen on the very edge of the cleft down which it must plunge, so that its course is perfectly straight from the beginning. Very often, however, this division is postponed until both nuclei are creeping among the bases of the stigmatic hairs ; in that case,

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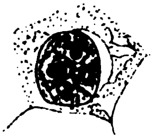
[1] Guignard, l. c., p. 177.



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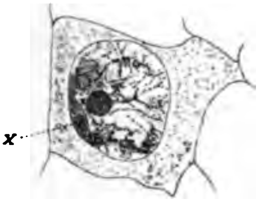

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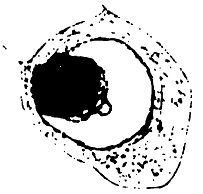

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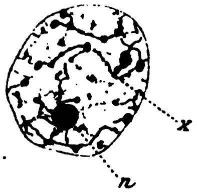

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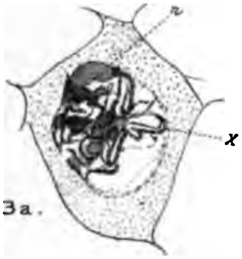

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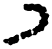

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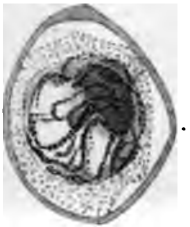

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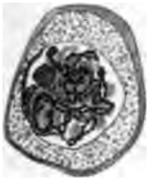

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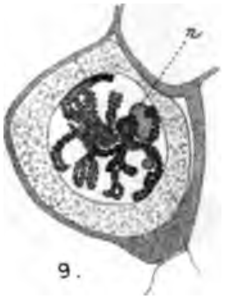

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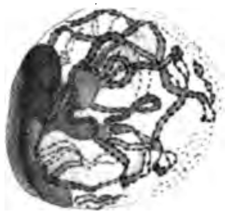

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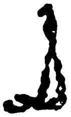

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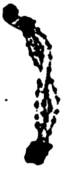

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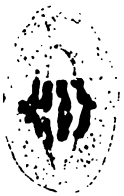

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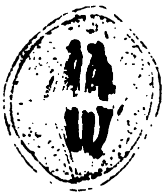

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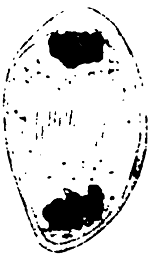

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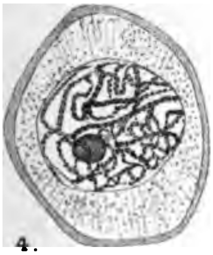

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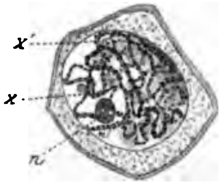

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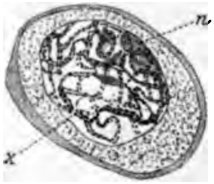

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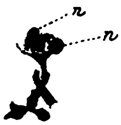

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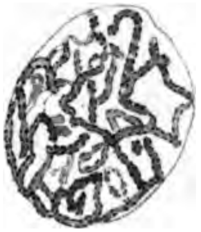

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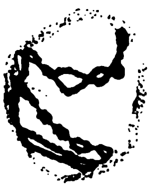

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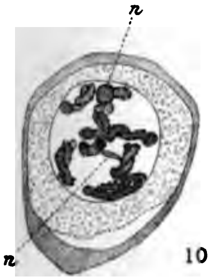

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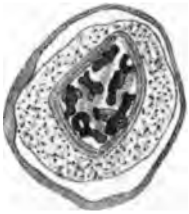

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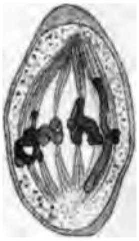

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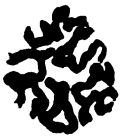

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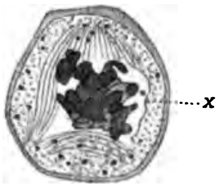

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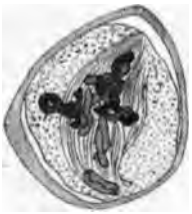

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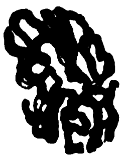

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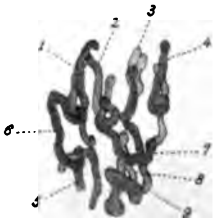

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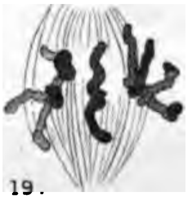

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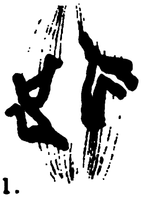

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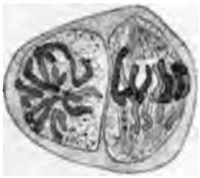

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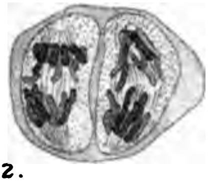

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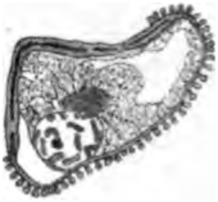

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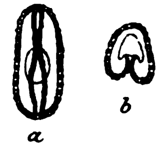

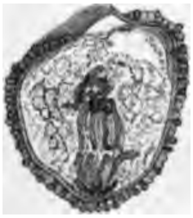

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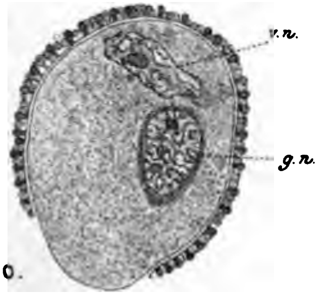

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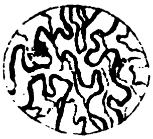
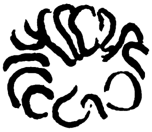
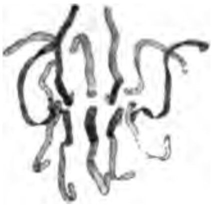
Homotype.	A 	B 	C 
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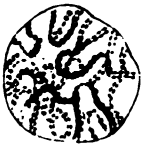
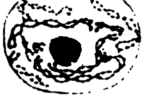
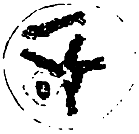
Heterotype.	G 	H 	I 
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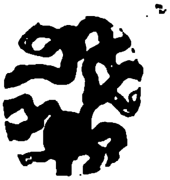

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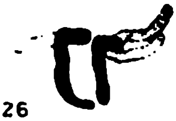

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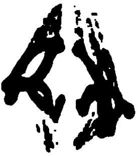

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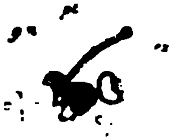

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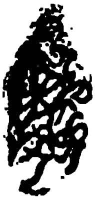

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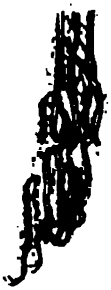

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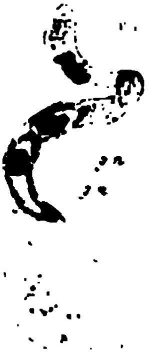

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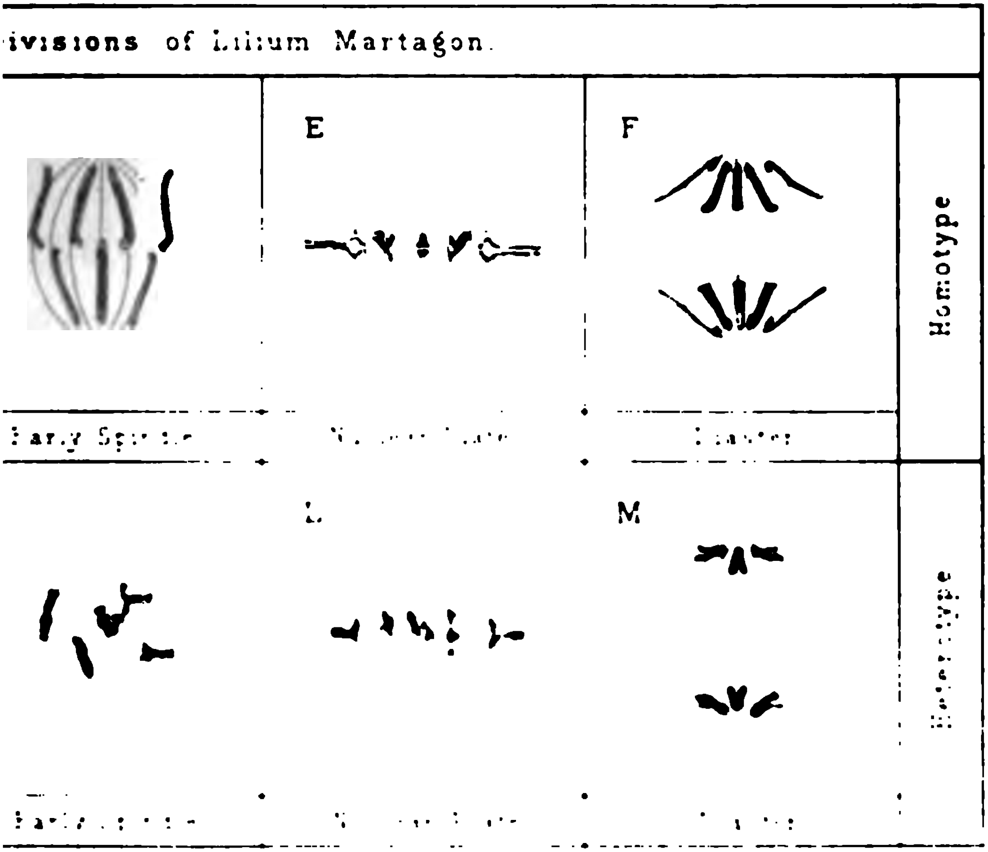

A Monograph of the Geoglosseae.

BY

GEORGE MASSEE, F.L.S.

Principal Assistant (Cryptogams), Royal Herbarium, Kew.

With Plates XII and XIII.

MORPHOLOGY AND REPRODUCTION.

THE amount of histological differentiation is very slight throughout the entire group: parenchymatous tissue, resulting from the complete union on every surface of hexagonal, more or less isodiametric cells, so characteristic of the 'cortex' or external stratum of the ascophore in other groups of the Discomycetes, is entirely absent from the members of the Geoglosseae; and in some of the most primitive species, as *Geoglossum hirsutum*, there is no clear line of demarcation between the fertile or ascigerous portion of the ascophore and the sterile or stem-like base. The stem in all known species is elongated and somewhat slender, and consists of a bundle of slender, transversely septate, usually unbranched hyphae, which are parallel and densely packed together at the periphery, and become lax and more or less interwoven at the centre. If a transverse section of a young stem is examined under the microscope it will be seen that the component cells separate readily from each other if the cover glass is moved laterally, whereas in a

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mature stem the peripheral cells are bound together by a ground-work of mucilage derived from the gelatinization of the outer layer of the cell-walls. The axial cells of the stem, and also of the ascigerous portion, usually become completely resolved into mucilage, which remains in the cavity thus produced. In dry weather the plant is fairly rigid, owing to the persistent hyphae of the 'cortical' or peripheral portion being cemented together by the indurated mucilage, whereas in rainy weather the entire fungus is lax and slimy, the dissolved mucilage dripping away in some species. The velvety or squamulose surface of the stem present in some species is due to the outgrowth of the free ends of the ascending peripheral hyphae (Fig. 86).

The hyphae of the stem pass without any modification to form the terminal club-shaped or pileate ascigerous portion, which is more or less hollow or spongy towards the centre, the hyphae branching and forming a densely interwoven peripheral layer or hypothecium, the numerous free ends of which either grow out into asci containing the spores, or continue as vegetative hyphae known as paraphyses. The component cells of the hypothecium show protoplasmic continuity very distinctly (Fig. 53). The asci and paraphyses, closely packed side by side, form the superficial layer or hymenium, which covers the entire surface of the fertile portion of the ascophore. In *Geoglossum hirsutum* and its several varieties, there are present in the hymenium, in addition to the asci and paraphyses, numerous black, pointed, rigid hyphae which resemble porcupine-quills when seen under the microscope; these spines project beyond the general level of the asci, and are also present on the stem, giving the entire fungus a minutely velvety appearance. When quite young these spines are hyaline and sparsely transversely septate; as growth proceeds the cell-wall becomes black, opaque, and rigid (Figs. 10 and 31 *a*).

These spines are morphologically identical and homologous with the bodies called *metuloids* by Cooke[1], that are met with in the hymenium of the lowest types of the Basidiomycetes, as *Hymenochaete*, where, as in *Geoglossum*, the spines are rigid and coloured, and project above the general level of the hymenium, giving it a velvety appearance, and possibly exercising a protective function in preventing the surface of the hymenium from being eaten by minute snails, &c., as is the case with the unprotected hymenium of the allied genera *Corticium* and *Stereum*. In *Peniophora*, a genus closely allied to *Hymenochaete*, the spines are colourless, not so rigid and the surface of the exposed portion when old is usually incrusted with particles of oxalate of lime. From this type of structure of the spines we pass on by a gradual transition to what are known as *cystidia*; large sterile cells of various shapes, present in the hymenium of many species of the Agaricineae. The primitive spine-like form of structure present in *Geoglossum* also occurs in the simplest known types of other groups of Fungi, as the Hydneae, Clavarieae, &c.

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[1] Grevillea, VIII, 20, 1879.

The asci are elongated and narrowly clavate, the apex somewhat narrowed, thick-walled, and furnished with an apical plug of a substance which becomes coloured clear blue when treated with a solution of iodine (Fig. 11). According to Boudier's[1] system of classification, the fleshy Discomycetes are divided into two primary groups depending on the mode of dehiscence of the asci. The Geoglosseae belong to the *Inoperculés*, characterized by the asci opening at the apex by a small circular opening having a raised, torn margin, or by an elongated slit, the rupture being effected by the swelling of the apical plug that becomes blue with iodine (Fig. 64). In the second group, called the *Operculés*, dehiscence takes place in a circumscissile manner, the apex of the ascus separating along a clearly defined line, like a lid, which either falls completely away, or remains attached by one side, looking like an upraised, hinged lid after dehiscence. The Ascoboleae are typical examples of the *Operculés*.

There are normally eight spores in an ascus; in the lowest types these are, as is usual throughout the Fungi, large and coloured, also multiseptate and arranged in a parallel fascicle in the ascus—this is the condition of things in *Geoglossum*; in *Spathularia* the spores are of the same type of structure, but hyaline or colourless. In *Mitrula* the spores are much smaller, hyaline, having few, or in some species no septa, and are arranged in two rows in the ascus. Finally, in the monotypic genus *Neolecta*, from Brazil, the spores are minute, globose, hyaline, and arranged in a single row in the ascus. In many species the spores are ejected elastically at maturity, but are often prevented from being diffused at once by the slime present on the hymenium. In the aquatic species, *Vibrissea truncorum*, the spores protrude from the ascus when mature, and are dispersed by the water.

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[1] Bull. Soc. Myc. France, I. 97, 1885.

No species belonging to the Geoglosseae has, so far as is at present known, a conidial form of reproduction.

All the species are saprophytes, growing on the ground or on dead leaves, &c., in damp places, and some few species are aquatic.

Bearing on the vexed question of sexual reproduction in the Discomycetes, the researches of Tulasne[1] on *Ascobolus furfuraceus* and *Peziza melaloma*, Woronin[2] on the structure and development of *Ascobolus pulcherrimus*, and De Bary[3] on *Peziza* (*Pyronema*) *confluens*, are well known, as is also the refutation of these respective statements by Brefeld[4], who denies the presence of sexual organs of functional value in the entire group. Van Tieghem[5] has also brought some strong evidence to bear against De Bary's sexual theory. Finally, Dangeard[6] has quite recently discovered what he considers to be an undoubted method of sexual reproduction, present not only in the Ascomycetes, but in the Fungi generally. The constant characteristics of this mode of sexual reproduction are said to be as follows:—(1) the existence of distinct gametes; (2) the fusion of nuclei; (3) the determinate number of bipartitions of the sexual nucleus.

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[1] Ann. Sci. Nat., Sér. V, Tom. VI (1866).

[2] Beitr. z. Morphol. und Physiol., Pilze, Heft II.

[3] Morphol. und Physiol., Pilze, 162–164.

[4] Bot. Unters. über Schimmelpilze.

[5] Traité général de Botanique, ed. II, pp. 1132 and 1166.

[6] Le Botaniste, IV, 21 (1894).

This mode of sexual reproduction, so far as the Ascomycetes are concerned, was first clearly observed by Dangeard in *Peziza vesiculosa*, Bull., and may be briefly summarized as follows:—

The stroma or hypothecium gives origin to the asci and paraphyses; the latter are simply unmodified filaments of mycelium, and require no special notice; on the other hand, each ascus owes its existence to the presence of an oospore, which originates as follows:—

Two filaments approach each other until they are in contact at the tips. the apical cell of each contains a single nucleus; these are the two gametes, male and female respectively; the apical cells of these gametes anastomose or conjugate, their protoplasm mixes, and the two large nucleolate nuclei fuse almost immediately, forming the oospore, which occupies the extremity of two conjugating filaments. Finally the apex of the oospore elongates and grows into an ascus, into which the nucleus ascends and by repeated bipartition produces a nucleus for each spore contained in the ascus (Fig. 92).

Immediately following the above statement. Dangeard adds, as a rider. that in addition to the production of the oospore by the conjugation of two distinct gametes. he has observed that in *Peziza vesiculosa* the oospore is sometimes formed by the fusion of the contents of two adjacent cells of the same hypha. this admission, although speaking volumes for the unbiassed disposition of the author, rather clashes with one of the three fundamental constants of his theory of sexual reproduction, viz. the existence of distinct gametes.

I have examined *Peziza vesiculosa*, and also other species belonging to the Discomycetes, and have been able to confirm Dangeard's statements relating to the origin of the asci, both from the conjugation of two independent hyphae and also from a single hypha, but at the same time I regret to say that I cannot accept his interpretation of the process for the following reasons:—

During a microscopic examination of the species of *Peniophora* and *Hymenochaete* undertaken some years ago, I was very much struck by repeatedly observing the metuloids or spine-like hymenial appendages to be furnished with a bifurcate base. Failing to offer a satisfactory explanation of this curious structure, the matter passed from my mind until the appearance of Dangeard's figures illustrating the conjugation of his gametes. Returning to the subject, I found that in *Peniophora velutina* the spines certainly did originate from the fusion of two distinct hyphae, but the conjugation occurs deep down in the interwoven substance of the subiculum, and is altogether too minute to show with certainty the fusion of the nuclei to form Dangeard's so-called oospore. It may here be stated that Cooke has, years ago, described and figured[1] the metuloids of *Peniophora* with a bifurcate base. I have also ascertained that the spines in the hymenium of *Hymenochaete*, *Veluticeps*, and *Geoglossum hirsutum* originate from the fusion of two hyphae. Considering, as already stated, that the gigantic cells known as *cystidia*, present in the hymenium of numerous species belonging to the Agaricineae, are homologous with the metuloids of *Peniophora* and *Geoglossum*, these were examined, and in *Coprinus atramentarius* showed very clearly the whole of the process as described by Dangeard, the approach of two independent hyphae until their apical cells were in contact, the fusion of these two cells, also of the two nuclei, finally the growth of the gigantic cell or cystidium from the oogonium, and the wandering of the nucleus into the cystidium (Fig. 91).

This peculiar structure of the cystidia being supported on two 'gametes' has also been previously recorded and figured by W. G. Smith[2], who writes as follows:—'In conclusion, I will advert to the way in which the cystidia in *Gomphidius* are borne. In many instances, if not in all, they arise from two conjoined cells as at J. I have not seen basidia so arise, and it looks superficially like what is termed conjugation.'

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[1] Grevillea, VIII, 21, Pl. 125, f. 16, 1879.

[2] Ibid. X, 79, Fig. J., 1881.

It may be argued that cystidia and metuloids, being hymenial appendages, are degraded basidia or asci, and thus their origin from conjugating filaments does not throw doubt as to the sexual nature of the process. To meet such a possibility, evidence of conjugation, illustrating all the details of the process as described by Dangeard, can be observed on a grand scale in the formation of the large hairs covering the exterior of the ascophore in species of *Ascobolus*, *Lachnea*, *Humaria*, &c. Now, in selecting protective hairs as evidence of the broadly distributed mode of formation of organs by conjugation, I think such cannot, by any amount of ingenuity, be considered as aborted spore-producing structures, but as purely vegetative parts of the ascophore. Figs. 93, 94, and 95 illustrate the mode of origin of the hairs from the ascophore of *Lachnea albo-spadicea*, Phillips; using the terms employed by Dangeard, the parts of the figures indicated would stand as follows: *a*, gametes; *b*, oospore (not complete in Fig. 93, as the two nuclei have not yet fused); *c*, the hair, which is a direct outgrowth of the oospore. Probably any species of *Lachnea* or pilose *Ascobolus* would give the same results, as the gigantic spinulose, thick-walled hairs borne by these species have the base furcate. The reader is referred to the numerous figures of hairs from the Discomycetes given in Cooke's Mycographia, showing the furcate base. Finally, the fungus called *Ciliaria bicuspis*, by Boudier[1], is so named on account of the bicuspid or forked base of the large hairs present on the margin and outside of the ascophore.

The above observations show that the coalescence of the apical cells of two distinct hyphae does not prove, in all cases, that these hyphae are gametes, in the usual sense in which that term is employed. Secondly, that the coalescence of two cells, the mixing of their protoplasm, and the fusion of their nuclei, does not necessarily constitute an oospore;

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[1] Bull. Soc. Myc. France, XII, [illegible], pl. III, f. 1, 1896.

and that under certain conditions, where these two conditions are fulfilled, a purely vegetative structure is produced as the result of such conjugation; consequently there is no evidence to prove that the conditions described by Dangeard as constituting a sexual act in the formation of the asci in the Ascomycetes are such in reality.

In some of the largest hairs present on the species of *Lachnea* it is not unusual for three 'gametes' to fuse at the tips to form the 'oospore,' and during the increase in size of the hair growth is often very unequal in the swollen basal portion, or 'oospore,' which frequently results in the supports or 'gametes' being separated from each other, and carried, apparently for some distance, up the hair, which then presents the appearance of a stem with three root-branches starting at slightly different levels. Owing to local growth at the basal portion of the hair, even when only the usual two 'gametes' are present, these not unfrequently eventually stand at different levels, and as the hair increases in size and becomes thick-walled, the 'gametes' also become indurated and coloured.

The most successful preparations for demonstrating the points of structure described above were obtained by first placing portions of the gills of a very young *Coprinus*, or the entire young plants of *Lachnea*, for four hours in a saturated solution of corrosive sublimate, to which a trace of acetic acid is added. After quickly washing in tap-water, the material is placed in absolute alcohol, where it can remain for an indefinite time. Sections are first placed in a very slight aqueous solution of nigrosin containing 4 per cent. of acetic acid, and afterwards transferred to a stronger solution of nigrosin alone; here they may remain for twenty-four hours, when they should be examined, and again replaced, if the nuclei are not sharply differentiated. The material would probably be best put up in balsam; mine were spoiled as permanent preparations by mounting in equal proportions of glycerine and water, with a trace of carbolic acid.

The above observations seem to suggest the idea that when specially large organs, such as asci, cystidia, or hairs, have to be produced, a usual method of effecting this is by the amalgamation of the apical cells of two independent hyphae.

The recent researches of Harper[1] on the sexual mode of reproduction in *Sphaerotheca Castagnei* tend to show that the observations of De Bary bearing on the question of sexuality in the Ascomycetes were in the main correct; nevertheless Harper's more detailed account rather upsets the generally accepted idea that the Ascomycetes are derived from the Phycomycetes, he having shown that in *Sphaerotheca*—the genus which more especially led De Bary to entertain this view—after fertilization of the carpogenic cell, a series of superposed cells is formed, and from one of these, and that not the terminal one, the ascus is formed; hence the ascus in *Sphaerotheca* is not directly homologous with the oogonium of the Phycomycetes.

It is the tendency at present to endeavour to trace groups presenting a general morphological resemblance to a common origin; nevertheless, the large group of organisms described by Thaxter[2] under the name of Laboulbeniaceae are morphologically characteristic Ascomycetes; yet the universal sexual mode of reproduction in this group by non-motile antheridia and trichogynes is essentially Floridean in character, and difficult to reconcile with the sexual process as described by Harper in *Sphaerotheca*, also a typical Ascomycete, and seems to necessitate the admission that what we at present term Ascomycetes will prove to be a heterogeneous assemblage, and that the single bond of agreement the production of ascospores does not necessarily indicate a unity of origin of the members possessing this feature.

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[1] Die Entwickelung des Peritheciums bei Sphaerotheca Castagnei, Ber. d. Deutsch. Bot. Gesellsch. Bd. XIII. Heft 10, 475, 1895.

Ueber das Verhalten der Kerne bei der Fruchtentwickelung einiger Ascomyceten, Pringsh. Jahrb. 1896, 655, Taf. XI–XII.

[2] Contribution towards a Monograph of the Laboulbeniaceae, Mem. Amer. Acad., XII. No. III, 1896.

AFFINITIES.

The constant characteristics of primitive types of Fungi in the various families of both Ascomycetes and Basidiomycetes are (1) comparative absence of differentiation of the sporophore, and (2) large coloured spores, the latter being often also multiseptate and the epispore more or less ornamented. The genus *Geoglossum* possesses these features in a very pronounced form; the ascophore is a simple, erect, more or less club-shaped structure, the upper portion of which is fertile, the thinner basal portion sterile, and called the stem. In *G. hirsutum*, the simplest form known, the transition from the sterile to the fertile portion is not indicated externally; in the other species there is a very slight ridge at the commencement of the ascigerous portion. The spores in all the species are large, brown, and multicellular; but there is a gradual decrease in the size of the spores and in the number of component cells from *G. hirsutum*, where the spores are almost as long as the ascus, and consist of fourteen cells, to the *G. glutinosum* group, in which the spores are shorter, and generally four-celled. In all the species of *Geoglossum* every part of the plant is black externally.

Following directly we have the genus *Mitrula*, the black species of which are externally quite indistinguishable from the species of *Geoglossum*, but are supposed to be generically distinct on account of the somewhat shorter, hyaline spores, which in the simplest forms are often four-celled, and in the highest forms have become very small and one-celled. In *Mitrula* the terminal ascigerous portion becomes more sharply defined from the stem, and is also often brightly coloured, every portion being covered by the hymenium. The next genus in the sequence of evolution is *Leotia*, which agrees with *Mitrula* so far as the spores are concerned, but differs in the form of the ascigerous portion, which is more or less pileate, or shaped like the cap of an Agaric, the margin curving inwards towards the stem. If we imagine the hollow, erect, fertile portion in *Mitrula* (Figs. 54, 59, 60) pressed down from the apex, we get the pileate form of *Leotia*; and in some of the higher forms of *Mitrula* this condition sometimes occurs, thus illustrating the so-called transition phases between those groups of species possessing several features in common, which we call genera.

The spores in *Spathularia* are of the *Geoglossum* type, but colourless; whilst the ascigerous portion is more sharply differentiated from the stem than in *Geoglossum*, and is also frequently laterally compressed or spathula-shaped, more especially in the species on which the genus is founded, whence the generic name. Finally, *Vibrissea* has the *Spathularia* type and colour of spore, but the ascigerous portion is pileate, thus differing from *Spathularia* in the same manner that *Leotia* differs from *Mitrula*.

From the above remarks it may possibly be inferred that genera in the Fungi are somewhat shadowy, but in reality not more so than in other branches of the Vegetable Kingdom, and perhaps our knowledge of true affinities becomes more exact in proportion as our belief in genera as entities becomes less.

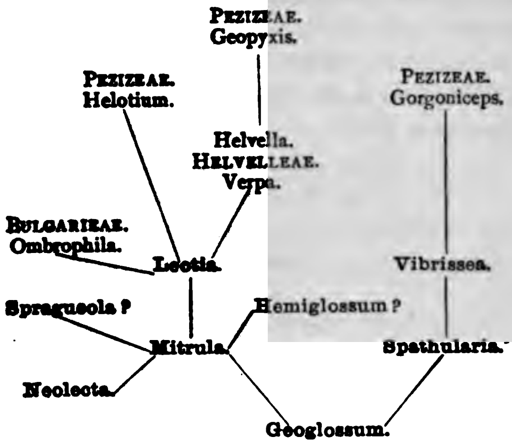
The transition from the Geoglosseae to other families of the Discomycetes is gradual; the line between species of *Leotia* and *Ombrophila* belonging to the Bulgarieae is purely a matter of personal opinion; the same may be said of the species of *Vibrissea* and *Gorgoniceps*, the latter belonging to the Pezizeae. *Leotia* shades into *Verpa* belonging to the Helvelleae on the one hand, and on the other into *Helotium*, belonging to the Pezizeae Finally, *Helvella* of the Helvelleae leads very gradually into *Geopyxis*, belonging to the Pezizeae.

The diagram on p. 236 shows the evolution of the genera of the Geoglosseae from *Geoglossum*; also the origin of the Helvelleae, Bulgarieae, and Pezizeae from the Geoglosseae.

DISTRIBUTION

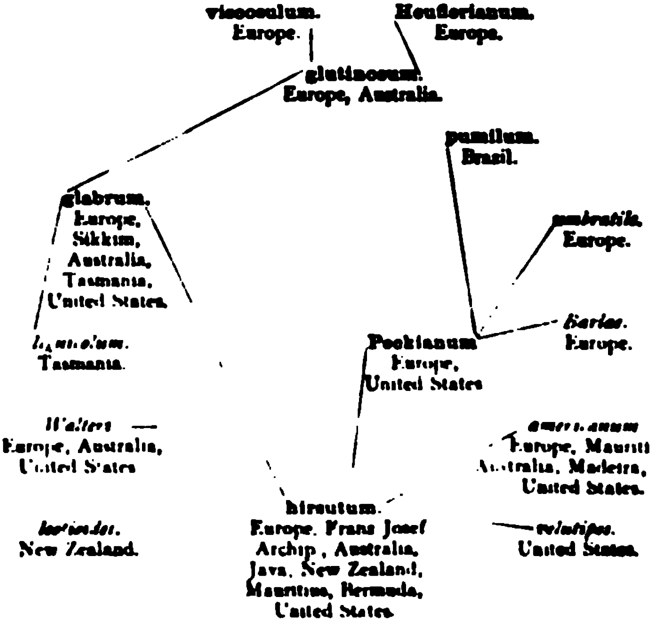
In dealing with problems relating to distribution, mere numbers are apt to mislead, owing to our comparatively unequal knowledge respecting the floras of different countries, and this imperfection is especially pronounced with regard to Fungi, which are, as a rule, altogether neglected by collectors, and even by botanists having no special interest in the group.

In the Geoglosseae however, fortunately, the general morphology and comparative differentiation of the species supports the numerical evidence bearing on the origin and gradual dissemination of the family. The Geoglosseae represent a primitive type of the Discomycetes, which as an order is comparatively modern compared with the remainder of the Ascomycetes, the Hysteriaceae, Sphaeriaceae, Tuberaceae, &c.; and the entire evidence points to Northern Europe as being the cradle of this interesting family. An analysis of the distribution of *Geoglossum*, the most primitive genus included in the family, will serve as a model, the remaining large genera following along similar lines. The following points stand out very clearly:—(1) The primitive and oldest genera have the most closely allied species, and even these are connected by forms and varieties. (2) Varieties and forms become more abundant the further the species extend from the original home of the genus.



Geoglossum contains seven species, six of which occur in

Europe, the seventh—*G. pumilum*—being recorded only from Brazil. *G. hirsutum*, the most primitive species in the genus, is represented in the Kew Herbarium, in the typical form, from the following places:—Franz Josef Archipelago, Europe, Mauritius, Java, Australia, New Zealand, Bermuda, United States. This species has four forms or varieties, only two of which—*Walteri* and *americanum*—occur in Europe, where both are very rare, whereas both are not uncommon in those distant parts of the world where the type-form is rare. Of the two remaining forms—*leotioides* and *velutipes*—the former is confined to New Zealand and the latter to the United States, and in both these countries the type-form is again rare. The following diagram shows the distribution of the species of *Geoglossum*, the species being printed in clarendon type, the forms in italics:—



In the present work the group Geoglosseae includes eight genera; but of these, *Spragueola*, a genus of my own founding, is far from being stable, and its one species may possibly prove to belong to the older genus *Mitrula*. Again, it is somewhat doubtful as to whether the genus *Hemiglossum* belongs to the present family; its only species suggests affinity with *Midotis*, but as I have had no opportunity of verifying this or otherwise, I accept the affinity indicated by its founder, Patouillard. Of the remaining six genera, with a total of fifty-eight species, five genera have their head-quarters in Europe, and include forty species. The extra-European genus *Neolecta*, including one species, is confined to Brazil. The distribution of species throughout the world is shown in the following table:—

N. Hemisphere	55 species.
S. "	9 "
Old World	47 "
New World	23 "

Endemic species are distributed as follows:—

Europe	27 species.
Greenland and Kamtschatka	3 "
China	3 "
Cape of Good Hope	1 "
United States	8 "
Brazil	2 "
Patagonia	1 "

The distribution of the genera and species are given in detail in the systematic section.

FAM. GEOGLOSSEAE.

Ascophore stipitate, erect, ascigerous portion terminal, clavate, spathulate, or pileate; asci elongated, narrowly clavate, 8-spored, dehiscing by an apical pore; spores coloured or hyaline, septate or continuous; paraphyses present.

GEOGLOSSEAE, *Sacc.*, Syll. VIII (as a subfamily of Helvelleae).

Most closely allied to the Bulgarieae, agreeing in the more or less gelatinous nature of the ascophore, but differing in not having a distinct plane or concave disk. The Pezizeae are also closely allied through the genus *Helotium*; the latter however again differs in the discoid hymenium and in the more or less parenchymatous cortex of the ascophore. *Verpa*, connecting the Helvelleae, differs in having the ascigerous portion more distinctly separated from the stem, and in the hymenium being sharply limited to its upper surface.

KEY TO THE GENERA.

I. *Spores elongated, arranged in a parallel fascicle in the ascus.*

* Spores coloured.

Geoglossum.

** Spores hyaline or colourless.

Spathularia. Ascigerous portion spathulate or clavate, laterally compressed.

Vibrissea. Ascigerous portion pileate, free margin incurved towards the stem.

II. *Spores narrowly elliptical, 1-2-seriate in the ascus.*

* Hymenium covering every part of the ascigerous portion.

† Ascigerous portion stipitate.

Mitrula. Ascigerous portion clavate, compressed.

Leotia. Ascigerous portion pileate.

†† Ascigerous portion sessile.

Spragueola.

** Ascigerous portion flat, hymenium covering only one surface, the other surface sterile.

Hemiglossum.

III. *Spores globose.*

Neoleota.

GEOGLOSSUM, *Persoon.*

Ascophore simple, erect, clavate, entirely black, sometimes with a tinge of olive or purple; ascigerous portion terminal, either gradually passing downwards into the stem, or its lower termination more or less indicated by a slight constriction. Asci clavate, apex narrowed, pore blue with iodine; spores 8, long and slender, septate, brown, arranged in a parallel fascicle in the ascus; paraphyses present; black, spine-like cystidia are also present in the hymenium of some species.

Geoglossum, Pers., Obs. Mycol. i, 11, 1796; emended by Saccardo, Consp. Gen. Disc., Bot. Central. xviii, 214, 1884.

Trichoglossum, Boud., Bull. Soc. Myc. Fr. i, 110, 1885, in part.

Cibalocoryne, Hazsl., Ung. Akad. d. Wiss. xi, 8, 1881, in part.

Clavaria, of old authors.

Most nearly allied to *Mitrula*, the black species of which differ in the hyaline, 2-seriate spores. Certain species of *Clavaria*, belonging to the Basidiomycetes, and *Xylaria*, belonging to the Pyrenomycetes, closely resemble the species of *Geoglossum* in general appearance and habit.

Distr.—The seven species included in the present genus have both individually and collectively a much wider range than those belonging to any other genus in the Geoglosseae. Six of the species—*G. hirsutum*, *glabrum*, *glutinosum*, *Peckianum*, *viscosulum*, and *Heuflerianum*—occur in Europe, the two last-named not occurring elsewhere. *G. pumilum* is peculiar to Brazil. The genus ranges from Franz Josef Archipelago to New Zealand, and is more frequent in warm regions than other genera; species being recorded from Cuba, Bermuda, Brazil, Java, and Sikkim. No species, so far as I am aware, have been found in China or Japan.

KEY TO THE SPECIES.

I. *Spines present in the hymenium.*

hirsutum.

II. *Spines absent from the hymenium.*

glutinosum. Plant 3–6 cm. high; spores almost cylindrical; paraphyses abruptly piriform or globose at the tips.

glabrum. Plant 3–7 cm. high; spores narrowly clavate; paraphyses clavate at the tip, constricted at the septa.

Peckianum. Plant 4–7 cm. high; paraphyses longer than the asci, tips curled.

pumilum. Plant up to 9 mm. long; spores 15-septate.

viscosulum. Ascigerous portion pitted or alveolate.

Heuflerianum. Entire plant 3–4 mm. high; spores 3-septate.

Geoglossum hirsutum, *Persoon*, Comment. Fung. Clav. 37, 1797; *Pers.*, Syn. Fung. 608, 1801; *Fries*, Syst. Myc. i, 488, 1821; *Cooke*, Mycogr. 3, f. 3, 1875; *Phillips*, Brit. Disc. 34, pl. 2, f. 9, 1887; *Sacc.*, Syll. viii, n. 150, 1889; *Massee*, Brit. Fung.-Fl. iv, 492, 1895; *Rehm*, Kr.-Fl. n. 5876, 1896. (Pl. XII, Figs. 31, 31 *a*, 32.)

Gregarious or caespitose; entire fungus 4–7 cm. high, black, dry, everywhere densely velvety; ascigerous portion [illegible] the length of the entire fungus, lanceolate, ovate, oblong, or almost globose, generally more or less compressed and longitudinally wrinkled, sometimes irregular in form and crooked, densely velvety, due to the projection of closely packed, pointed black hairs (cystidia), up to 1·5 cm. broad, scarcely or not at all differentiated from the equally velvety stem. Asci clavate, apex narrowed, pore blue with iodine, 160–220 × 18–20 μ; spores 8, arranged in a parallel fascicle in the ascus, linear, almost cylindrical, ends very slightly narrowed, multiguttulate, then 7- and finally 15-septate, often slightly curved, 110–150 × 5–6 μ, light umber brown, translucent; paraphyses slender, septate, tips brown, clavate, curved, slightly longer than the asci; cystidia or spines numerous, straight, pointed, black and opaque, paler towards the base, narrowly fusiform, 8–10 μ thick.

Syn. *Clavaria simplex hirsuta*, Schmidel, Icon. Plant. 92, tab. xxv, 1762.

Clavaria ophioglossoides, Sowerby, Eng. Fung. tab. 83, 1797.

Geoglossum hirsutum β capitatum, Pers., Syn. Fung. 608, 1801.

Trichoglossum hirsutum, Boudier, Bull. Soc. Myc. i, 110, 1885.

Exs. [illegible], *Schmidt and Kunze*, Deutschl. Schw., n. cxxii; *Moug. and Nest.*, Stirp. Crypt. n. 94; *Crépin*, Herb. Bot. Belge, n. 1084; *Roum.*, Fung. Gall. n. 4043; *Rabenh.*, Fung. Eur. n. 523; *Klotzsch-Rabenh.*, Herb. Myc. n. 44 and 237; *Sydow*, Myc. March. n. 440 and 1069; *Fuckel*, Fung. Rhen. n. 1141; *Allescher and Schnabl*, Fung. Bavar. 352; *Cooke*, Fung. Brit. n. 393; *Desm.*, Crypt. France, sér. 1,

n. 420; *Karsten*, Fung. Fenn. n. 451; *Roumeg.*, Fung. Gall. Select. n. 63; *Vize*, Micro.-Fung. n. 481.

Hab.—On damp ground among grass, &c.

Distr.—Britain, Ireland; Portugal (*Henriques*); France; Denmark; Sweden; Germany; Holland; Belgium; Austria; Switzerland; Italy; Finland; Mauritius (*Telfair*); Bermuda (Devonshire marsh, *Challenger Exp.*); New Zealand (*Colenso*); Java; Victoria (*Müller*); United States (Sandlake, N.Y., *Peck*; New England; Massachusetts; Louisiana, *Hale*, n. 3661); Franz Josef Archipelago (Cape Flora, alt. 60 ft., Jackson-Harmsworth Polar Exp., coll. *Fisher*).

The present species, in its typical form, is readily known by the densely velvety texture of every part of the fungus. The typical number of septa in the spore is 15, but not unfrequently there are fewer in some of the spores of a plant having as a rule the normal number, and in one instance I have seen two of the spores in an ascus with 19 septa each, the remaining six having the usual 15 septa.

Persoon's var. *capitata* is not a constant variety, nor even a constant form; it was established by Persoon upon two of the figures in Schmidel's plate which occur in a long series showing the variation of form of the ascigerous portion, which passes gradually from the most usual, lanceolate, through ovate, shortly clavate, obovate, and subglobose.

Form **americanum.**

Spores 100–125 × 5–6 μ; usually 7–9-septate, pale brown; cystidia or spines scarcely longer than the asci, hence the hymenium appears to be almost glabrous when seen under a lens.

Syn.—*Geoglossum hirsutum*, var. *americanum*, Cooke, Mycogr. 3, fig. 1, 1875.

Geoglossum americanum, Sacc., Syll. viii, n. 151, 1889.

Hab.—On the ground among grass, &c.

Distr.—Britain (Penzance; Glasgow, Bot. Garden; Twycross, *Berkeley*); France (*Desm.*, Crypt. France, sér. i, n. 420, some of the specimens); Germany (*Holl, Schmidt, and Kunze*, Deutschl. Schwämme, xcvii, some of the specimens; *Mougeot and Nestler*, Stirpes Crypt. 94, also 95, the lower specimen; Ratzeburg, *Rudolphi*); Hungary (*Thümen*, Fung. Austr. n. 927); Madeira (*Lowe*); Cuba (*Wright*); Mauritius; Victoria; Queensland; United States (N. York, *Gerard*, n. 53; Louisiana, *Hale*).

Differs mainly from the typical form in the reduced length of the spines, and in the somewhat shorter spores with fewer septa, 7–9 being the most frequent numbers, although it is not unusual to find odd spores with a greater number of septa; I have seen a spore with 15 septa in the same ascus with seven others having 9 septa each, in Cooke's type of var. *americanum*.

Form Walteri. (Pl. XIII, Figs. 78–80.)

Spores 60–75 × 5–6 μ pale brown, most frequently 3–5-septate, but occasionally 7–9-septate; spines scanty, not at all, or only very little, longer than the asci.

Syn.—*Geoglossum Walteri*, Berk., in Cooke, Mycogr. 4, fig. 4 (which is altogether inaccurate); Sacc., Syll. viii, n. 149, 1889.

Geoglossum Farlowi, *Cooke*, Grev. xi, 107, 1883; *Sacc.*, Syll. viii, n. 148, 1889.

Hab.—Among grass and on trunks of tree-ferns.

Distr.—Britain (Twycross, *Bloxam*), United States (Newton, Mass., *Farlow*), Australia (Wild Dog Creek, Apollo Bay).

This condition may be considered as a continuation of the reduction in length of spines, and size and number of septa of the spores, commenced in form *americanum*. In the present form the spores are most frequently 3-septate.

Cooke first suggested *G. Walteri* as a species to Berkeley, the latter having considered it as *G. hirsutum*, whereupon Berkeley gave the specific name of *Walteri*, leaving Cooke to draw up a diagnosis of the species. Judging alone from the figure and description given in Mycographia (4, f. 4), the form appears to have no close affinity with *G. Farlowi*, Cke., but I have carefully examined the type, and find that Cooke has drawn the spines too long—they are in reality not much longer than the asci; the spores are also too long, and the 3-septate form is most usual.

Following the diagnosis of *G. Farlowi* (Grev. xi, 107), Cooke says, 'It is very much a matter of opinion whether this (*G. Farlowi*) and *G. Walteri*, C., and *G. velutipes*, Pk., should be regarded as varieties of *G. hirsutum*, or as distinct species.'

Form velutipes.

Subcaespitose, black; club [= ascigerous portion] short, compressed; stem densely clothed with a very black, velvety pubescence. Asci lanceolate; spores fasciculate, at first simple or triseptate, then elongated and 9–11-septate, brown, ·002′–·005′ long [=about 50–120 µ]; paraphyses septate, recurved at the tips.

Syn.—*Geoglossum velutipes*, Peck, 28th Rep. N. Y. State Mus. 65, 1875, figured on pl. 1, f. 16–19, of the 29th Report; Sacc., Syll. viii, n. 152, 1889.

Hab.—Ground in hemlock woods.

Distr.—United States (Oneida, Northville).

This species is easily distinguished, both by its somewhat caespitose habit and its very black, hairy stem. The difference between the young and the mature spores is quite noticeable. I have not seen specimens of *G. Walteri*, a hairy species from Australia; but as it is said to have the spores 7-septate, it must be distinct from our plant.

The above is a reproduction of Peck's description of the species. The author depends too implicitly upon the number of septa present in the spore, which is indeed a most fallacious character. I have had no opportunity of examining specimens, and it is uncertain as to which of the forms it is most closely allied. The subcylindrical spores and paraphyses recurved at the tips prove it to be a condition of *G. hirsutum*. The figures are represented as bristling with black spines all over, but in the description the stem alone is described as velvety.

Form **leotioides**. (Pl. XII, Figs. 8–12.)

Gregarious, black, entire fungus 1·5–2·5 cm. high, every part velvety, with projecting, spine-like, black cystidia; ascigerous portion globose or broadly ovate, hollow, at length collapsing and drooping round the stem as in *Leotia*, up to 1 cm. diameter; stem erect, equal, straight, 1·5–2 cm. high, about 2 mm. thick. Asci almost cylindrical, apex narrowed, pore blue with iodine, about 230 × 20 µ; spores 8, arranged in a parallel fascicle, dingy umber, translucent, 17-septate at maturity, linear-cylindrical, or very slightly thickened above the middle, ends a very little narrowed, usually slightly curved, 150–165 × 5 µ; paraphyses slender, straight throughout, septate, tip slightly clavate, umber, almost straight, spines acute, black and opaque, numerous, longer than the asci.

Syn.—*Geoglossum hirsutum*, var. *leotioides*, Cooke, Grev. viii, 61, 1879; Sacc., Syll. viii, n. 150, 1889; Cke., Hdbk. Austr. Fungi, 251, 1892.

Hab.—On the ground.

Distr.—New Zealand (Wellington, *Travers*).

Superficially resembling Persoon's capitate or globose form of *G. hirsutum*, but clearly distinguished by the longer spores and the almost straight paraphyses.

Geoglossum glutinosum, *Pers.*, Obs. Myc. i, 11, 1796; *Cke.*, Mycogr. 5, f. 6, 1875; *Phil.*, Brit. Disc. 38, 1887; *Sacc.*, Syll. viii, n. 136, 1889; *Massee*, Brit. Fung.-Fl. iv, 490, figs. 8–10 on p. 188, 1895; *Rehm.*, Kr.-Fl. n. 5871, f. 7, p. 1145, 1896. (Pl. XIII, Figs. 66, 67.)

Gregarious, entire fungus 3–6 cm. high, black, sometimes with a tinge of olive or brown, more or less viscid; ascigerous portion lanceolate, glabrous, terete or compressed, 5–8 mm. broad, usually about ½ the length of the entire fungus; stem cylindrical, rather slender, viscid, often brownish black. Asci subcylindrical, apex narrowed, pore blue with iodine, 210–240 × 12–14 μ; spores 8, arranged in an imperfectly parallel fascicle in the ascus, or inclined to become irregularly 2–3-seriate, linear, almost cylindrical, ends obtuse, pale brown, translucent, generally straight, for a long time 3-septate, sometimes passing to 5–7-septate; paraphyses slender, septate; tips brown, varying from abruptly piriform to globose, 7–9 μ diameter.

Syn.—*Geoglossum viscosum*, Pers., Comm. Fung. Clav. 38, 1797; Cooke, Mycogr. 8, f. 10, 1875; Phil., Brit. Disc. 37, 1887; Sacc., Syll. viii, n. 137, 1889; Massee, Brit. Fung.-Fl. iv, 490, 1895; Rehm, Kr.-Fl., n. 5870, 1896.

Geoglossum glutinosum, forma *minor*, Sacc., Mich. i, 444, 1877; Sacc., Syll. viii, under n. 136, 1889 (ascophore hardly 1 cm. long).

Geoglossum Mulleri, Cooke, Mycogr. 4, f. 2, 1875 (spores and paraphyses incorrectly represented); Sacc., Syll. viii, n. 138, 1889.

Exs.—*Karsten*, Fung. Fenn. n. 450; *Roumeg.*, Fung. Gall. n. 4044; *Mougeot and Nestler*, Stirp. Crypt. n. 780; *Desm.*, Crypt. France, sér. 1, n. 422; *Klotzsch-Rabenh.*, Herb. Myc., ed. ii, n. 319.

Hab.—On the ground among grass, moss, &c.

Distr.—Britain, Portugal (*Henriques*), France, Sweden, Belgium, Switzerland, Germany, Austria, Italy, Finland, Australia (Port Philip, *French*; Melbourne, *Muller*, *Reader*).

The chief characteristics of the present species, as defined above,

are: the glabrous, more or less viscid ascophore; narrow, almost cylindrical asci, and nearly cylindrical, comparatively short spores with obtuse ends, which usually remain 3-septate. The spores are often much paler in colour than in the other species; and, owing to their shorter length, do not form such a distinct parallel fascicle in the ascus, but are inclined to become 2–3-seriate. In going over a large series of specimens, I find it impossible to separate *glutinosum* from *viscosum*. The relative amount of viscidity depends on the weather; the terete or grooved stem depends on age, the last condition depending on the deliquescence and gelatinization of the central portion of the ascophore, which again depends on external conditions. The two extreme forms of the tips of the paraphyses, as figured by Cooke, look distinct and of value as aids to specific identification, but every possible transition from broadly piriform to globose can be met with. The spores usually remain 3-septate, but sometimes pass on to 5–7-septate. In one specimen in Karsten's Fung. Fenn. n. 450, the spores are entirely normal in size and shape, deep brown, and apparently quite mature, but show no traces of septation. On the other hand, the specimens in Roumeguere's Fung. Gall. Exs. n. 4044, while the majority of the spores are 3-septate, some have 5 septa and others are quite distinctly 7-septate, the spores in the last-named instances not being above the normal size.

The form *minor* of Saccardo appears only to differ in its small size, and every mycologist who has done field work knows how general it is to meet with specimens belonging to almost every species of fungus that might be dubbed forma *minor* or *major*, as the case might be; but every one knows that such forms are by no means permanent, but akin to the stunted crop of wheat grown during a very dry season. Finally, I have examined the type of *G. Mülleri*, Cooke, and find that it agrees with *G. glutinosum* in all essential features; the sizes of the spores, as given by Cooke in Mycographia, are too large, and the paraphyses are in reality capitate.

Geoglossum glabrum, *Persoon*, Obs. Myc. ii, 61, 1799; *Pers.*, Syn. Meth. Fung. 608, 1801; *Fries*, Syst. Myc. i, 488, 1821; *Cooke*, Myc. 7, f. 10, 1875; *Phil.*, Brit. Disc. 36, 1887; *Massee*, Brit. Fung.-Fl. iv, 491, 1895. (Pl. XII, Figs. 44–46.)

Gregarious; entire fungus 3–7 cm. high, dry, black or brownish-black, sometimes tinged with olive or purple; ascigerous portion ⅓–½ the entire length of the fungus, not sharply differentiated from the stem below, glabrous, cylindric-clavate or variously deformed, 3–8 mm. broad, sometimes laterally compressed; stem rather slender, minutely squamulose, often wavy. Asci cylindric-clavate, apex narrowed, pore blue with iodine, 165–210 × 18–20 μ; spores 8, arranged in a parallel fascicle in the ascus, elongate-clavate, straight or very slightly curved, clear brown at first, then dark smoky brown, 75–85 × 8–9 μ, 7-septate, very slightly constricted at the septa; paraphyses septate, becoming gradually clavate upwards for the upper half, septa often rather close together near the apex, and constricted at the septa, giving a moniliform appearance, brown, 7–9 μ thick at the apex, which may be straight or slightly curved.

Syn.—*Clavaria ophioglossoides*, Linn., Sp. Pl., ed. i, v, 2, p. 1182, 1753 (fide Saccardo).

Geoglossum ophioglossoides, Sacc., Syll. viii, n. 141, 1889; Rehm, Krypt.-Flora, n. 5872, 1896.

Geoglossum sphagnophilum, Ehrb., Sylv. Myc. 30, 1818; Sacc., Syll. viii, n. 142, 1889.

Geoglossum ophioglossoides, var. *sphagnophilum*, Rehm, Krypt.-Flora, n. 5872, 1896.

Geoglossum australe, Cooke, Mycogr. 6, f. 8, 1875; Sacc., Syll. viii, n. 144, 1889.

Geoglossum simile, Peck, 25th Report N.Y. State Mus. 97, 1872 (in the 29th Rep., Peck himself says this species is identical with *G. glabrum*).

Exs.—*Holl, Schmidt, and Kunze*, Deutschl. Schw. n. xcviii; *Ellis and Everh.*, N. Amer. Fung. sér. 2, n. 2031; *Jack, Leiner, u. Sitzenberger*, Krypt. Badens. n. 55; *Moug. and Nest.*, Stirpes Crypt. 684, also the upper specimen in n. 95 of the same authors (the lower specimen is *G. glabrum*, form *americanum*, Cooke); *Phil.*, Elv. Brit. Fasc. ii, n. 55; *Rehm*, Ascom. n. 503 *a* and *b*; *Roum.*, Fung. Select. Gall n. 262; *Klotzsch-Rabenh.*, Herb. Myc. n. 238; *Karsten*, Fung. Fenn. n. 452; *Fuckel*, Fung. Rhen. n. 1142; *Sydow*, Myc. March. n. 285; *Rabenh.-Winter*, Fung. Eur. n. 2845.

Hab.—On the ground among grass, &c.

Distr.—Britain, Ireland, Portugal, Spain, France, Belgium, Switzerland, Germany, Denmark, Sweden, Finland, Russia, Sikkim (*Dr.* [now *Sir*] *J. D. Hooker*), Tasmania (*Archer*), Victoria, Queensland, United States.

The specific characteristics of the present species are: the dry, black ascophore, distinctly clavate spores, very slightly constricted at the septa, and the paraphyses becoming clavate upwards, where the septa are rather close together and constricted, giving a moniliform appearance; in some specimens, however, the septa are rather distant from each other near the apex of the paraphyses, which are not so strongly moniliform, whereas in others this appearance is altogether lost, and every transition may often be seen, even in the same specimen, from one extreme of paraphysis type to the other. The most constant feature of the species is the distinctly clavate form of the spores.

The above specific diagnosis is drawn up from a specimen in the Kew Herbarium called *Geoglossum glabrum* by Persoon himself; hence we are certain that we are so far correct. The specimens in all the exsiccati quoted have been examined, and agree with Persoon's fungus. I cannot accept Saccardo's decision that Persoon's fungus is synonymous with *Clavaria ophioglossoides*, Linn. The diagnosis of this species by Linnaeus (Sp. Pl., ed. i, vol. ii, p. 1182, 1753) is as follows: 'Clavata integerrima compressa obtusa,' and this I consider as inadequate. I have gone through the Herbarium of Linnaeus, and find nothing to support Saccardo's idea. The fact that Bulliard, Bolton, and others have called a species *ophioglossoides* proves nothing; in many cases, in the absence of specimens, we do not know what species, as at present understood, these authors had in view. On the other hand, where the name *ophioglossoides* (Linn.) is used in exsiccati, we find that it covers several species, including the hyaline spored *Mitrula microspora* of the present work, as in *Desm.*, Crypt. France, sér. 1, n. 421. Finally, Schmidel (Icon. Plant. 92, 1762) considers that the fungus Linnaeus had in view was Schmidel's *Clavaria simplex hirsuta* (= *G. hirsutum*, Pers.).

Form difforme.

Ascigerous portion often bent or irregular in form, compressed, obtuse, slightly viscid when moist; paraphyses septate, apex slightly thickened and often more or less swollen below the septa, curved or slightly wavy.

Syn.—Geoglossum difforme, Fries, Obs. Myc. i, 159, 1815; Cooke, Mycogr. 6, fig. 7, 1875; Sacc., Syll. viii, n. 143, 1889; Massee, Brit. Fung.-Flora, iv, 492, 1895; Rehm, Kr.-Fl. n. 5873, 1896.

Geoglossum nigritum, Cooke, Mycogr. 205, fig. 345, 1879; Sacc., Syll. viii, n. 145, 1889; Rehm, Kr.-Fl. n. 5874, 1896.

Clavaria nigrita, Fries, Hymen. Eur. 676, 1874, not of Persoon, Syn. 604.

Exs.—*Cooke*, Brit. Fung. n. 481; *Cooke*, Brit. Fung. ed. ii, n. 394; *Berk.*, Brit. Fung. n. 256; *Roumeg.*, Fung. Gall. n. 2419; *Klotzsch-Rabenh.*, Herb. Myc. n. 424.

Hab.—On the ground among grass, &c.

Distr.—Britain, Ireland, France, Belgium, Germany, Sweden, Finland, Italy, Austria, Victoria (Harkaway Range, *French*), South Carolina.

Specimens from Fries in the Kew Herbarium show that his *G. difforme* and *G. nigritum* are identical, and again in a broad sense are identical with *G. glabrum*, differing mainly in the tips of the paraphyses not being so distinctly clavate, and constricted at the septa near the apex. The characteristic clavate form of the spore is present.

As *Geoglossum nigritum* may be again restored by some one to specific rank, its true history may as well be told, so as to prevent complication in the future. Persoon described a fungus, which he called *Clavaria nigrita* (Pers., Syn. 604, 1801). There seems every reason to believe that this fungus was a genuine *Clavaria*, as that genus is defined at the present day, and that the description and figure given by Bresadola (Fung. Trident. 62, tab. lxvii, fig. 4) represents Persoon's fungus. Fries quotes Persoon's fungus as *Clavaria nigrita*, Pers., and gives Persoon's own diagnosis of his species in Syst. Myc. i, 483, 1821, and Epicr. 578, 1836–38. In Hym. Eur. 676, 1874, Fries again gives Persoon's name and diagnosis of the fungus under consideration, but adds, 'species insignis, habitu Geoglossi, nuperius ad Halmbyboda prope Upsaliam lecta (v. v.).' Here, for the first time, Fries collected what he supposed to be the *Clavaria nigrita* of Persoon. A portion of this gathering was sent by Fries to Berkeley, and is now in the Kew Herbarium. This specimen was examined by Cooke, and found to be a *Geoglossum*, and figured, inaccurately, in Mycogr. 205, fig. 345, 1879. Cooke, however, did not doubt this fungus being the *Clavaria nigrita*, Pers., and figured it as such, on the faith of the species determined by Fries, calling it '*Geoglossum nigritum*, Pers.'

Var. lignicolum. (Pl. XII, Figs. 1, 2.)

Gregarious on rotten wood, 4–5 cm. high, entirely black with a decided purple tinge; ascigerous portion narrowly clavate, about ⅓ the entire length, about 3 mm. thick, round or compressed, becoming imperfectly hollow, glabrous, dry, passing gradually into the slender, usually crooked, equal, minutely velvety stem. Asci clavate, apex rounded and tinged blue with iodine, often curved, 150 × 15 μ; spores linear-clavate, apex thickest, brown, translucent, usually very slightly curved, 7-septate, arranged in a parallel fascicle in the ascus, 8 in number, 80–85 × 5–6 μ; paraphyses straight, septate, clavate, apex tinged olive and about 6 μ thick.

***Syn.*—*Geoglossum lignicolum*, Massee, Journ. Bot. xxxiv, 145, pl. 357, f. 19–20, 1896.**

***Hab.*—On rotten wood.**

***Distr.*—Tasmania (*Archer*).**

Growing on rotten wood along with the type specimen of *Mitrula vinosa*, Berk., which it resembles superficially.

Having again examined the present fungus, I come to the conclusion that it must be reduced to a variety of *G. glabrum*, differing mainly in the purple tinge of the ascophore, and the less distinctly moniliform paraphyses. The spores are of the characteristic *glabrum* type.

Geoglossum Peckianum, *Cooke*, Hedw. 1875, p. 10; *Cooke*, Mycogr. 5, f. 5, 1875; *Sacc.*, Syll. viii, n. 147, 1889. (Pl. XII, Figs. 42, 43.)

Gregarious or tufted, black, stem viscid, entire fungus 4–7 cm. high, glabrous; ascigerous portion narrowly lanceolate, somewhat compressed, narrowing below into the stem, but the termination distinctly defined, less than half the length of the entire fungus; stem very minutely squamulose, viscid. Asci narrowly elliptic-fusiform, apex narrowed, pore blue with iodine, about 180–200 × 18–20 μ; spores 8, arranged in a parallel fuscicle in the ascus, clear brownish-umber, translucent, linear-fusoid or subclavate, ends slightly narrowed, sometimes slightly curved, 7-septate at first, finally 15-septate at maturity, 115–125 × 6–7 μ; paraphyses slender, septate, longer than the asci, tips brownish, slightly thickened, variously curved and twisted.

Syn.— *Geoglossum glutinosum*, Peck, 25th Rep. N. Y. State Mus. 97, 1872, not of Persoon.

Hab.—Swampy ground.

Distr.—United States (Sandlake and Forestburgh, N.Y., *Peck*; Gainsville, Fla., *Ravenel*, n. 89; New England, *Murray*, n. 5339; Lower Carolina, *Curtis*, n. 1227), England (a specimen from Sowerby's herbarium, now at Kew, called *Geoglossum difforme*), France (Montmorency, *Boudier*).

Distinguished among the glabrous species more especially by the paraphyses, which are longer than the asci, and strongly curved or spirally twisted at the thickened, coloured tips. Neglecting the spines, this species is almost indistinguishable from some forms of *G. hirsutum*.

Form **umbratile.**

Scattered or gregarious, elongato-clavate, 3–6 cm. high, 2–3 mm. thick, glabrous, even, longitudinally striate when dry, black. Asci cylindrical, very shortly stipitate, apex obtuse, 160 × 20 μ; spores 8, 2–3-seriate, rod-shaped-fusoid or somewhat clavate, 7-septate, not constricted, dusky, 80 × 5 μ; paraphyses slender, 4 μ thick, tips pale brown, strongly circinate.

Syn.—*Geoglossum umbratile*, Sacc., Mich. i, 444, 1879; Sacc., Syll. n. 146, 1889.

Hab.—Swampy ground.

Distr.—Italy (Bizzozero).

As suggested by Saccardo, the present differs from *G. Peckianum*, mainly in the 7-septate spores.

Form **Barlae.**

Blackish-olive, 3–5 cm. high; ascigerous portion compressed, glabrous, somewhat tongue-shaped, 1–2 cm. broad; stem paler, blackish furfuraceous at the apex. Hymenium blackish-olive, even, not at all or scarcely viscid in rainy weather, well defined from the stem. Asci fusiform-clavate, base narrowed and wavy, 300–320 × 18–20 μ; spores 8, umber, linear-clavate, straight or slightly curved, 7-septate, 85–95 × 6–7 μ; paraphyses brownish, simple or branched at the base, septate, apex incrassated, torulose.

Syn.—*Geoglossum Barlae*, Boudier, Soc. Mycol. France, iv, 76, 1888; Sacc., Syll. viii, in a note under n. 146, 1889.

Hab.—On clay ground among grass.

Distr.—France (Nice, *Barla*).

Again, Boudier considers the present form to be distinguished from *G. Peckianum* more especially by the 7-septate spores. The blackish-olive, broader ascigerous portion is the only tangible distinction between the present and form *umbratile*.

Geoglossum pumilum, *Winter*, Grev. xv, 91, 1887; *Sacc.*, Syll. viii, n. 153, 1889.

Small, blackish; ascigerous portion ovate or somewhat deformed, capitate, distinct, slightly and irregularly compressed, perfectly smooth, up to 3 mm. long, and apparently viscid; stem subcylindrical, often slightly compressed and grooved, up to 6 mm. long, furnished with squarrose fascicles of brownish hairs. Asci cylindric-clavate, sessile, 8-spored, 230–255 × 25–27 μ; paraphyses filiform, expanding upwards into a thick brownish apex up to 10 μ broad, sometimes septate towards the apex, straight; spores cylindrical, slightly narrowed and rounded at both ends, somewhat curved, generally 15-septate, very slightly constricted at the septa, brown, 94–110 × 7 μ.

Hab.—On clay ground.

Distr.—Brazil (Sao Francisco, Prov. Sta. Catharina, *Ule*, n. 338).

The above is Winter's diagnosis of the species.

Geoglossum viscosulum, *Sacc.*, Syll. viii, n. 140, 1889.

Black, viscid, glabrous; ascigerous portion gradually attenuated into the stem, the fertile surface divided into quadrate areolae; these areolae are convex when moist and concave when dry. Asci clavate, apex narrowed; spores 8, cylindrical, brown, 3–5-septate, somewhat shorter than the ascus; paraphyses septate, apex capitate straight or curved.

Syn.—*Cibalocoryne viscosula*, Hazsl., Ung. Akad. d. Wiss. xi, 8, 1881.

Hab.—On the ground among moss.

Distr.—Hungary (*Hazslinszky*).

Apparently a very distinct species, although the diagnosis unfortunately leaves something to be desired. The areolation of the hymenial surface is the most tangible feature mentioned, and, if constant, will afford an easy means for distinguishing the species, or may even prove to be of generic value, thus forming a connecting link with the *Morchella* group; but until the species is better known, it is perhaps best in *Geoglossum*, as placed by Saccardo. A more or less alveolate condition of the hymenium occurs in *Mitrula Rehmii* and *M. muscicola.*

Geoglossum Heuflerianum, *Bail*, Herb. Mycol. typ. n. 180 *b* (without a diagnosis); *Sacc.*, Syll. viii, n. 139, 1889; *Rehm*, Krypt.-Flora, n. 5869, 1896.

Very small, clavate, black, 3–4 mm. long; spores terete-fusoid, rounded at both ends, almost straight, 45–50 × 10 μ, 3-septate, dusky; paraphyses moniliform at the apex.

Hab.—Among moss.

Distr.—Mutters, Tirol.

Remarkable for the very small size of the ascophore, and the 3-septate, thick spores. The above is the diagnosis given by Saccardo (Syll. viii, n. 139).

SPATHULARIA, *Persoon.*

Erect, stipitate; ascigerous portion obovate, spathulate, or elliptical, more or less hollow, laterally compressed, glabrous, decurrent down opposite sides of the stem, from which it is sharply differentiated. Asci clavate, apex narrowed; spores 8, arranged in a parallel fascicle in the ascus, elongated filiform-clavate, hyaline, multiguttulate then multiseptate; paraphyses present.

Spathularia, Persoon, Tent. Disp. meth. Fung. 36, 1797; Fries, Syst. Myc. i, 490, 1821; Sacc., Syll. viii, 48, 1889; Massee, Brit. Fung.-Flora, iv, 485, 1895; Rehm, Krypt.-Flora (Discom.), 1158, 1896.

Spathulea, Fries, Plantae Homon. 86, 1825. 'Spathularia; quod nomen paululum mutandum ob animal homonymon' (Fries, l. c.).

Clavaria, *Elvella*, *Mitrula*, and *Leptoglossum* in part, of authors.

Closely resembling the genus *Mitrula* in general appearance and habit, but in the last-named the spores are much shorter and broader, and 2-seriate in the ascus. The only other genus having very long, linear spores arranged in a parallel fascicle in the ascus is *Vibrissea*, but here the ascigerous portion is pileate or horizontal, the margin free and incurved; the upper surface covered with the hymenium, the under surface sterile. In *Spathularia* the ascigerous portion is adnate to the stem throughout, and every part is covered with the hymenium.

Distr.—A small genus of six species, confined to the North Temperate Zone. *S. rufa*, *S. Neesii*, *S. nigripes*, and *S. clavata* occur in Europe; the last-named species also occurs in the United States, while *S. flava* is confined to the last-named country.

KEY TO THE SPECIES.

I. *Stem white or yellow.*

clavata. Ascigerous portion broadly obovate.
flava. Ascigerous portion narrowly elliptic-oblong.

II. *Stem dark-coloured.*

* Stem minutely velvety.
velutipes.
** Stem glabrous.
nigripes. Stem violet-brown.
rufa. Ascigerous portion obovate, margin even, powdered with lilac meal.
Neesii. Ascigerous portion spathulate or sphaeroid, margin wavy.

Spathularia clavata, *Sacc.*, Mich. ii, 77, 1882; *Sacc.*, Syll. viii, n. 160, 1889; *Massee*, Brit. Fung.-Flora, iv, 485, figs. 22–24 on p. 188; *Rehm*, Krypt.-Fl. n. 5877, figs. 1–4 on p. 1146, 1896. (Pl. XIII, Figs. 50–53.)

Gregarious or in small clusters, entire fungus 3–10 cm. high, dry; ascigerous portion spathulate or broadly clavate, obtuse, or sometimes more or less cleft at the apex, laterally compressed, surface wavy or somewhat lacunose, margin crisped or undulated, running down the stem for some distance on opposite sides, hollow, clear yellow, rarely with a tinge of red, 2–4 cm. high, 1–2·5 cm. broad; stem ½–1 cm. thick, often thickened at the base, hollow, white, often becoming yellowish. Asci clavate, apex narrowed, pore blue with iodine, 100–140 × 12–15 μ; spores 8, arranged in a parallel fascicle in the ascus, hyaline, filiform-clavate, base narrowed, multiguttulate then multiseptate, enclosed in a mucilaginous sheath, often slightly bent, 50–65 × 3 μ: paraphyses very slender, septate, often branched, the unthickened tips more or less curved.

Syn.—Elvella clavata, Schaeffer, Icon. Fung. ii. tab. 149, 1773.

Helvella spathulata, Afzelius, Vet. Acad. Handl., 1775.

Clavaria spathulata, Mull., Flor. Dan. tab. 658, 1775.

Helvella feritoria, Bolton, Hist. Fung. tab. 97, 1789.

Spathularia flavida, Persoon, Tentamen Disp. meth. Fung. 36, 1797; Cooke, Mycogr. 203, f. 342, 1878; Phil., Brit. Disc. 30, pl. ii, f. 7, 1887.

Helvella spathularia, Sowerby, Eng. Fung. tab. 97, 1789.

Spathularia flava, Swartz, Vet. Akad. Handl. 10, 1812.

Spathularia flavida, β crispa, Corda, Icon. ii, tab. 15, f. 125, 1838.

Mitrula spathulata, Fries, Summa Veg. Scand. 583, 1846.

Spathularia flavida, P., var. *plicata*, Thümen, in Thüm., Fung. Austr. n. 25.

Exs.—Sydow, Myc. March. n. 2516; Flor. Exs. Austro-Hung. n. 1947; *Ellis*, N. Amer. Fung. n. 1268; Schweiz. Krypt. n. 215; *Fuckel*, Fung. Rhen. nn. 1143, 2484; *Klotzsch-Rabenh.*, Herb. Myc. n. 28; *Roum.*, Fung. Sel. Gall. n. 326; *Karsten*, Fung. Fenn. n. 140; *Thümen*, Fung. Austr. n. 925; *Jack, Leiner, u. Sitzenb.*, Krypt. Badens, n. 337; *Cooke*, Fung. Brit. n. 470; *Phil.*, Elv. Brit. n. 3; *Rab.*, Fung. Eur. n. 126, *Holl, Schmidt, and Kunze*, Deutschl. Schw. n. cxciv; *Rehm*, Ascom. n. 426; *West. and Wall.*, n. 1085; *Berk.*, Brit. Fung. n. 257; *Desmazières*, Crypt. France, sér. 2, n. 455; *Sacc.*, Myc. Ven. n. 168.

Hab.—Britain, Portugal (*Henriques*), France, Belgium, Norway, Sweden, Germany, Austria, Switzerland, Italy, Finland, United States (Maine, *Fuller*, n. 22).

Very variable in size, the smaller forms sometimes closely resembling large specimens of *Mitrula laricina*.

Spathularia flava, *Massee* (non Swartz). (Pl. XII. Figs. 25, 26.)

Gregarious; ascigerous portion narrowly elliptic-oblong or narrowly elliptical, laterally compressed, grooved, showing a tendency to be decurrent down opposite sides of the stem, from which it is sharply differentiated at the base, about 2 cm. long, 5–7 mm wide, pale primrose-yellow; stem equal, or slightly thickened at the summit, a little paler in colour than the fertile portion, and about equal to it in length. Asci clavate; spores arranged in a parallel fascicle, narrowly cylindrical, 8–10-septate, almost as long as the ascus; paraphyses not seen.

Syn.—*Geoglossum flavum*, Gillet, Disc. France, 24, with a fig., 1879.

Leptoglossum flavum, Sacc., Syll. viii, n. 159, 1889.

Hab.—Among moss.

Distr.—France.

Spathularia velutipes, *Cooke and Farlow*, Grev. xii, 37, 1883; *Farlow*, Appalachia, iii, 245, 1884; *Sacc.*, Syll. viii, n. 164, 1889. (Pl. XIII, Figs. 85–88.)

Gregarious, entire fungus 3–5 cm. high; ascigerous portion spathulate, even, margin somewhat wavy, laterally compressed, decurrent down opposite sides of the stem, hollow, 1·5–2 cm. high, 1–1·5 cm. wide, tawny yellow; stem hollow, nearly equal, minutely velvety, dark brown with an orange tinge. Ascus clavate, apex narrowed, not blue with iodine, 85–100 × 10–11 μ; spores 8, arranged in a parallel fascicle, filiform-clavate, apex only slightly thicker than the base, slightly curved, multiguttulate, 55–60 × 1·5 μ; paraphyses numerous, cylindrical, septate, not thickened at the strongly curved tips, 2 μ thick.

Exs.—*Ellis and Everh.*, N. Amer. Fung. ser. 2, n. 2029.

Hab.—On mossy trunks in damp woods.

Distr.—United States (White Mountains and Lake Willoughby, Vt., *Farlow*).

This species is common in the region of the White Mountains and Lake Willoughby, Vt., and has been found also in other States. It has generally been named by American collectors *Spathularia flavida*, but it differs widely from that species. The substance is firm and even tough, and specimens shrink comparatively little in drying. The fungus is seldom more than two inches high, and the stipe is of a dark velvety brown, while the hymenium is somewhat yellow, but never of the bright yellow colour of *S. flavida* (Farlow, l.c.).

A note on the label accompanying the specimens sent to Cooke by Professor Farlow reads as follows: 'This is a common *Spathularia* in the colder parts of New England, where it grows on mossy logs in places which are somewhat moist, but not very wet. The dried specimen gives a good idea of the habit and colour when fresh, as it changes very little in drying.'

Distinguished from all known species by the dark velvety stem. The spores are thinner than in *S. flavida*, and I have not been able to demonstrate the existence of an investing mucilaginous sheath; this however is best seen in fresh specimens, and is of no specific value.

Type specimen examined.

Spathularia nigripes, *Sacc.*, Syll. viii, n. 161, 1889.

Ascigerous portion ovoid-oblong, 1 cm. long, laterally compressed, rugose, undulate, yellowish or flesh-colour, apex rosy or rufescent; stem cylindrical or compressed, 1 cm. long, base inflated, slightly rugulose, glabrous, violet-brown; flesh soft, white, smell pleasant; spores acicular, 50 μ long, guttulate, hyaline.

Syn.—*Mitrula nigripes*, Quélet, Suppl. xi, 11, tab. vii, f. 3, Assoc. Franç., 1883.

Hab.—Caespitose or scattered in sandy woods.

Distr.—Maritime Alps (*Barla*).

Quélet suggested that the present species was allied to *Mitrula rufa*, but this resemblance is superficial only. Saccardo, on the other hand, thinks it may be *Spathularia crispata*, forma *spathulata* of Fuckel, Symb. Myc., Append. ii, 66. This again is meaningless, as no one has any idea as to what *S. crispata* is, since it was never described nor figured.

Spathularia rufa, *Swartz*, Vet. Akad. Handl. 1812, p. 11; *Cooke*, Mycogr. 204, fig. 343, 1878; *Sacc.*, Syll. viii, n. 162, 1889; *Rehm*, Kr.-Fl. n. 5879, 1896; not of *Nees*, Syst. 171, tab. xvii, f. 156 B. 11.

Gregarious, entire fungus 1–4 cm. high; ascigerous portion obovate, even, margin not crisped nor wavy, laterally compressed, decurrent down opposite sides of the stem, about 1 cm. high and 5–7 mm. broad, dingy rufous-orange, more or less powdered with lilac meal; stem terete, usually more or less thickened at the base, reddish brown or bay. Asci clavate, apex narrowed, not blue with iodine, 100–130 x 10–12 μ; spores 8, arranged in a parallel fascicle, filiform-clavate, apex thickest, straight or slightly curved, multiguttulate then multiseptate, 60–70 x 2 μ; paraphyses slender, septate, sometimes branched, tips often curved, about 2 μ thick.

Syn.—*Mitrula lilacina*, Quélet, Enchir. 269, 1886.

Exs.—Rabenh., Fung. Eur. n. 235 (called *Spathulea rufa*, Nees); *Roum.*, Fung. Gall. Exs. n. 2075.

Hab.—On the ground in woods and shady places.

Distr.—Germany, France.

Distinguished from allies by its smaller size, dingy orange-brown colour, and in having the ascigerous portion even, and more or less powdered with lilac meal. A very fine series of specimens of this species are in the Kew Herbarium, having the following on the paper on which they are mounted: '*Spathulea bulbosa*, Rud. Herb. Neuhoff, Mecklenburg-Strelitz, in pinetis. Dr. F. Rudolphi.'

Spathularia Neesii, *Bres.*, Fung. Trident. 66, tab. lxxii, fig. 3, 1881; *Sacc.*, Syll. viii, n. 163, 1889; *Rehm*, Kr.-Fl. n. 5878, 1896.

Ascigerous portion ovoid, sphaeroid, or spathulate, laterally compressed, decurrent down opposite sides of the stem, even or rugulose, margin wavy, gelatinous-fleshy, whitish tan then lurid ochraceous, somewhat egg-yellow when dry, 1·5–2 cm. high, 1–1·5 cm. broad; stem roundish or somewhat compressed, even, becoming rugulose, glabrous, ochraceous-fuscous, apex paler, stuffed, 1·5–2 cm. long, 3–4 mm. thick; flesh similarly coloured, inodorous, slightly acid. Ascus fusoid-clavate, not blue with iodine, 110–150 × 12–18 μ; spores filiform-clavate, apex thickest, multiguttulate, slightly curved, tinged yellow, 60-80 × 1·5–2·5 μ, with an outer mucilaginous coat, tinged yellow, 8 in an ascus, arranged in a parallel fascicle; paraphyses numerous, branched, filiform, tips curved, about 2 μ thick.

Syn.—Spathularia rufa, Nees, Syst. 171, tab. xvii, f. 156 B. (not of Swartz nor Cooke); Pers., Myc. Eur. i, 198; Gillet, Disc. France, 26, with fig.

Spathularia rufa, var. *badipes*, Pat. Tab. Anal. Fung. 70, f. 161.

Mitrula rufa, var. Quél. xi, Suppl. p. 19.

Hab.—On pine leaves, &c., densely gregarious.

Distr.—Germany, France, Italy.

Bresadola has shown that the fungus called *Spathularia rufa* by Nees is not the fungus so called by Swartz, and further considers that the fungus described above is the one Nees had in view. The above description and synonymy is from Bresadola.

VIBRISSEA, *Fries*, emend.

Stipitate, vertical; ascigerous portion pileate, horizontal, margin thick, incurved towards the stem, upper surface fertile, under surface sterile, glabrous; stem simple, central. Asci narrowly elongato-clavate, apex narrowed, pore not blue with iodine; spores 8, needle-shaped, hyaline, multiguttulate, sometimes becoming multiseptate, arranged in a parallel fascicle in the ascus; paraphyses very slender, sometimes branched and the tips curved, septate.

Vibrissea, Fries, Syst. Myc. ii, 31, 1823.

Leotia, Hill, Hist. Pl. 43, 1751, in part.

Cudonia, Fries, Summa Veg. Scand. 348, 1846, in part.

Helotium of old authors, in part.

Superficially resembling *Leotia* in the ascigerous portion or pileus being horizontal or pileate, with a free, incurved margin, and in having an elongated stem, but differing in having needle-shaped spores arranged in a parallel fascicle in the ascus. Several sessile or subsessile species that have been included in the present genus by different authors, are here excluded on account of the presence of a more or less distinctly parenchymatous cortex, and absence of the characteristic margin incurved towards the stem. *V. truncorum* is a truly aquatic fungus, and at maturity the needle-shaped spores escape partly from the asci and vibrate in the water, hence the generic name.

Distr.—A small genus containing four well-defined species confined to the North Temperate Zone. *V. truncorum* and *V. circinans* occur in Europe and the United States; *V. lutea* and *V. ochroleuca* are confined to the last-named country. Of the two doubtful species, *V. vermicularis* is recorded from European Russia, and *V. rimarum* from Kamtschatka.

KEY TO THE SPECIES.

I. *Stem minutely velvety or squamulose.*

truncorum. Pileus deep orange-red.

II. *Stem glabrous.*

circinans. Pileus pale yellowish flesh-colour, under surface wrinkled, the ridges running down the apex of the stem.

lutea. Pileus and stem yellow.

ochroleuca. Pileus ochraceous-white; stem white.

Vibrissea trunoorum, *Fries*, Syst. Myc. ii, 31, 1823; *Phil.*, Brit. Disc. 316, pl. x, f. 60, 1887; *Phil.*, Trans. Linn. Soc. (Bot.), ser. 2, ii, 5, pl. 1, f. 1–9, 1881; *Sacc.*, Syll. viii, n. 167, 1889; *Rehm*, Kr.-Fl. n. 5888, 1896; *Massee*, Brit. Fung.-Fl. iv, 487, figs. 32–35, p. 188, 1895. (Pl. XII, Figs. 15–17*a*.)

Gregarious or scattered, often in clusters of 2–3 specimens, entire fungus 1–2 cm. high; ascigerous portion rather fleshy, orbicular, slightly convex, margin thick, free, and inclined to be incurved, disk glabrous, even, usually clear orange-red, sometimes yellow or brownish red, minutely silky with the protruding spores at maturity, 3–5 mm. across; stem 6–15 mm. long, 1·5–3 mm. thick, round, almost equal throughout, densely covered with coloured obtuse-septate hyphae spreading at right angles to the stem; when these hyphae are arranged in clusters the stem appears to be minutely squamulose, varying in colour from white to pale grey, brownish, or with an olive tinge. Asci very long, narrowly cylindrical, apex slightly narrowed, not blue with iodine, 225–250 x 6 μ; spores 8, filiform, hyaline, multiguttulate then multiseptate, 200 x 1 μ, arranged in a parallel fascicle in the ascus; paraphyses very slender, sometimes branched, tips slightly thickened and often tinged yellow.

Syn.—*Leotia truncorum*, Alb. and Schz., Consp. Fung. Nisk. 297, tab. 3, f. 2, 1805; Pers., Myc. Eur. i, 199, 1822.

Leotia clavus, Pers., Myc. Eur. i, 200, tab. xi, fig. 9, 1822.

Vibrissea Margarita, White, Scottish Nat. ii, 218, 1874; Phil., Brit. Disc. 317; Phil., Trans. Linn. Soc. (Bot.), ser. 2, ii, 6, pl. 1, f. 10–16, 1881; Sacc., Syll. viii, n. 170, 1889.

Vibrissea truncorum, Fr., var. *albipes*, Peck, 44th Rep. N.Y. State Mus. 37, 1891.

Exs.—*Phil.*, Elv. Brit. n. 4 and 4 *bis*; *Roumeg.*, Fung. Sel. Gall. n 536; *Desm.*, Crypt. France, sér. 1, n. 830; *Moug. and Nest.*, Stirp. Crypt. n. 781; *Ellis*, N. Amer. Fung. n. 134.

Hab.—On decaying and submerged wood, branches, and leaves, principally in subalpine districts.

Distr.—England, Scotland, Wales, France, Germany, Italy, Hungary (*Hazslinszky*), Finland, Belgium, Switzerland (Gadmenthal, *G. Nicholson*), Portugal (*Henriquez*), United States (New England, *Sprague*; N. Jersey, *Ellis*; Cascade Mountains, *Dr. Lyall*).

A truly aquatic fungus, completing its entire development only when entirely submerged. and attaining the largest size and most brilliant colour when occurring in alpine streams. Very beautiful specimens were found by Mr. G. Nicholson, Curator of the Royal Gardens, Kew, in a small stream in the Gadmenthal, opposite Feldmoos, Switzerland, in September, 1896. Peck's var. *albipes* is nothing more than the typical form, which was originally described and figured by Albertine and Schweinitz as having a white stem. When removed from the water in which it grows, every part of the fungus becomes darker in colour.

Vibrissea circinans, *Hazsl.*, Rendh. Köggornbák, in Ung. Akad. d. Wiss. xi, 15, 1881. (Pl. XII, Figs. 13, 14.)

Gregarious, often growing in circles; ascigerous portion fleshy, somewhat viscid when moist, pileate, convex, becoming more or less irregular, margin incurved, usually wavy; under surface concave, minutely wrinkled, the ridges often running down the stem, glabrous, pale yellowish flesh-colour or yellowish, 1–2·5 cm. broad; stem 2–6 cm. long, often crooked, imperfectly hollow, cylindrical or thickened above or below, pallid or reddish, pulverulent, glabrous. Asci clavate, apex rather acutely narrowed, pore not blue with iodine, 150–180 × 10–12 μ; spores 8, arranged in a parallel fascicle, linear-clavate, often slightly curved, at first multiguttulate, then multiseptate, 50–60 × 2 μ; paraphyses filiform, sometimes branched, tips not thickened, often curved.

Syn.—*Leotia circinans*, Pers., Comm. Fung. Clav. 31, 1797; Pers., Icon. et Descr. 16, t. 5, f. 5–7, 1798–1800; Fries, Syst. Myc. ii, 27, 1823; Cke., Mycogr. 97, fig. 172, 1879; Phil., Brit. Disc. 24, pl. ii, f. 5, 1887.

Helotium circinans, Swartz, Vet. Akad. Handl. p. 15, 1812.

Cudonia circinans, Fries, Summa Veg. Scand. 348, 1846; Rehm, Kr.-Fl., n. 5887, 1896; Sacc., Syll. viii, n. 165, 1889.

Leotia gracilis, Pers., Myc. Eur. i, 198, 1722.

Helvella revoluta, Wahlb., Fl. Upsal. 464, 1820.

Exs.—*Rabenh.*, Fung. Eur. n. 38 and 312 (called *Podonia circinans* (*Pers.*), *Fries*, Mspt.). *Karst.*, Fung. Fenn. n. 153; *Thum.*, Myc. Univ. n. 1809; *Fckl.*, Fung. Rhen. n. 1139; *Roum.*, Fung. Sel. Exs. 4738.

Hab.—Among moss, &c., in woods, chiefly pine.

Distr.—Britain, France, Portugal, Sweden, Norway, Germany, Bohemia, Austria, Finland, Lapland, United States.

Most closely allied to *Vibrissea lutea*, Peck, under which species the two are compared.

Specimens from Persoon examined.

Vibrissea lutea, *Peck,* 25th Rep. N. Y. State Mus. 97, pl. i, figs. 19–23, 1872. (Pl. XII, Figs. 5–7.)

Gregarious, 1·5–2·5 cm. high; ascigerous portion subglobose, smooth, margin often lobed, inflexed, free, concave below, 4–8 mm. across, yellow; stem nearly equal, even, smooth, solid, longitudinally wrinkled when dry, rather deep yellow. Asci clavate, apex rather acutely narrowed, not blue with iodine, 115–130 × 12 μ; spores 8, arranged in a parallel fascicle in the ascus, filiform and slightly thickened near the apex, often multiguttulate, 80–90 × 1·5 μ; paraphyses numerous, delicately septate, not thickened at the strongly curved tips, hyaline, longer than the asci, about 2 μ thick.

Syn.—Cudonia lutea, Sacc., Syll. viii, n. 166, 1889.

Hab.—Prostrate, mossy trunks of trees and among fallen leaves in woods.

Distr.—United States (North Elba, *Peck*).

Specimen from Professor Peck examined. The spores often contain a row of oil-globules, and in some instances there are indications of septa.

Cooke, in Mycogr. 97, has quoted this species as a synonym of his *Leotia circinans* (*Vibrissea circinans*, Hazsl.); this however is a mistake, the present species being quite distinct in the longer spores and form and colour of the ascophore.

Vibrissea ochroleuca, *Massee.* (Pl. XIII, Figs. 70–72.)

Scattered, somewhat gelatinous, entire fungus 2·5–3 cm. high; ascigerous portion pileate, fleshy, undulate, margin involute, pale ochraceous, glabrous, up to 1 cm. across; stem about 2·5 cm. high and 3 mm. thick, glabrous, white, rather flexuous. Asci clavate, apex narrowed. not blue with iodine, narrowed below into a long, slender pedicel, 100–110 × 8–9 μ; spores 8, filiform, hyaline, slightly curved, at first 3-septate, then 5–7-septate, 25–30 × 2 μ, arranged in a parallel fascicle in the ascus; paraphyses very slender, septate, often branched, tips not thickened.

Syn.—Leotia ochroleuca, Cooke and Harkness, Grev. ix, 8, 1880; Sacc., Syll. viii, n. 2515, 1889.

Hab.—On damp ground.

Distr.—California (*Harkness*, n. 1371).

Most nearly allied to *Vibrissea circinans*, from which it is distinguished by the shorter spores and pure white stem.

Type specimens examined.

Doubtful Species.

Vibrissea vermicularis, *Weinmann*, Hym. Gast. Ross. 487, 1836.

Simple, capitulum suborbicular, sublacunose, watery-pallid; stem subterete, blackish-brown.

Gregarious, stipes firmly attached, replete with a gelatinous mass when young, becoming partly hollow with age, generally cylindrical (very rarely attenuated towards the base or compressed), straight or curved, tough, smoky-black, paler below the pileus. Pileus ½–2 lines broad, watery-pallid when young, then slightly tinged with blue.

Hab.—On damp rotten wood of *Alnus incana*?

Distr.—Russia.

Vibrissea rimarum, *Fries*, Syst. Myc. ii, 32, 1823; *Sacc.*, Syll. viii, n. 169, 1889.

Subfasciculate; yellow, head becoming tawny, stem compressed.

Allied to the previous one [*V. truncorum*], but truly distinct. Entire fungus, from the peculiar habitat, much compressed; stem 1 inch long, thickness variable, flexuous, somewhat connate at the base. Cap hemispherical, small in proportion, becoming rufous, otherwise the entire fungus is yellow (Fries).

Hab.—In cracks in old rotten worked beams.

Distr.—Kamtschatka (*Wormskiold*).

MITRULA, *Fries*, emend.

Ascophore erect, black or bright coloured, dry, or in some species slightly viscid when moist; ascigerous portion clavate, subspathulate, or globose, often laterally compressed, and showing a distinct tendency to become decurrent down opposite sides of the stem, from which it is sharply differentiated, glabrous; stem sometimes squamulose or pulverulent. Asci narrowly cylindric-clavate, apex narrowed or obtuse, pore blue with iodine in some species, not coloured in others; spores hyaline, narrowly elliptical, septate, rarely continuous, 2-seriate, rarely 1-seriate, paraphyses present.

Mitrula, Fries, Syst. Myc. i, 491, 1821.

Geoglossum, Pers., Obs. Myc. i, 11, 1796, in part.

Microglossum, Sacc., Consp. Gen. Disc., Bot. Centralb. xviii, 214, 1884, in part.

Leptoglossum, Cooke, Mycogr. 250 (as a subgenus), 1879; as a genus, by Saccardo, Consp. Gen. Disc., in Bot. Centralb. xviii, 214, 1884, in part.

Verpa, Swartz, Vet. Ak. Handl. 129, 1815, in part.

Clavaria, in part, by old authors.

The present genus closely resembles in general appearance and habit *Geoglossum* and *Spathularia*, but differs from both in the much shorter, hyaline, elliptical spores being arranged in a 2-seriate or 1-seriate manner in the ascus. *Mitrula laricina* often grows on heaps of dead leaves that are floating in water. *Leotia* has similar spores, but the ascigerous portion is pileate, with a free margin incurved towards the stem.

Distr.—The twenty-five species included in the present genus have collectively a wide area of distribution, extending from Greenland to Tasmania, although with one exception—*M. rufa*—being absent from the tropics. The following fifteen, which constitute more than half the known species, are, so far as is known, confined to Europe:—*M. purpurascens*, *Saccardoa*, *olivacea*, *lutescens*, *vitellina*, *pusilla*, *sphaerocephala*, *gracilis*, *sclerotipes*, *muscicola*, *Rehmii*, *multiforme*, *globosa*, *sclerotiorum*, *arenaria*. The following also occur in Europe and other countries:—*M. serpentina*, *laricina*, *cucullata*, *microspora*. The United States has six species, two of which—*M. elegans* and *luteola*—are not known from elsewhere. One species only is peculiar to the southern hemisphere—*M. Berterii*—having been collected in Juan Fernandez, Tasmania, and New Zealand.

KEY TO THE SPECIES.

I. *Pileus with a distinct purple tinge.*

purpurascens. Plant 4–6 cm. high; every part blackish or blackish-brown, with a distinct purple tinge.

Berterii. Plant about 2 cm. high, slender; every part dark coloured, with a purple tinge.

Saccardoi. Plant 7–8 cm. high; apex of pileus purplish, remainder of plant whitish.

II. *Pileus dark green or ochraceous-olive.*

serpentina. Entire fungus dingy green; stem minutely squamulose.

olivacea. Entire fungus dingy green; stem glabrous, base whitish.

(There is a purplish-coloured variety of this species.)

lutescens. Entire fungus pale ochraceous-olive except base of stem, which is green.

III. *Pileus clear yellow to orange.*

laricina. Pileus broadly ovate or subglobose, egg-yellow.

vitellina. Pileus cylindrical, apex narrowed, egg-yellow.

elegans. Entirely pale yellow; stem slender, elongated, glabrous.

luteola. Entirely pale yellow; stem short, distinctly tomentose.

IV. *Pileus tawny-yellow, brown, or bay.*

rufa. Entire fungus 4–6 cm. high; rufous to dingy yellow.

cucullata. Entire fungus 1–2 cm. high; head irregularly obovate, stem dark brown, crooked.

pusilla. Entire fungus ½–1 cm. high; head clavate or subglobose; stem yellow, usually crooked. Growing on dead leaves of *Pinus silvestris.*

sphaerocephala. Entire fungus under 1 mm. high; head globose, stem straight. Growing on dead pine leaves.

gracilis. Entire fungus 2–2·5 cm. high; head orange-brown; stem wavy, flesh-colour.

sclerotipes. Entire fungus rusty-yellow, 1–2 cm. high; stem springing from a small yellowish sclerotium.

muscicola. Entire fungus 1–1·5 cm. high; head subglobose, rugulose towards the base; stem yellow.

Rehmii. Entire fungus 2–3·5 cm. high; head obovate, with longitudinal ribs; stem white, then yellowish.

bicolor. Entire plant about 6 cm. high; head narrowly lanceolate, brown; stem wavy, channelled.

multiforme. Fungus 1–2 cm. high; pileus clavate, capitate, or pileate, dingy brown; stem whitish.

globosa. Plant 3–5 cm. high; head globose; stem slender, every part dark bay.

V. *Entirely whitish or pallid.*

sclerotiorum. About 1 cm. high; springing from a sclerotium.

VI. *Plant entirely black.*

microspora. Plant 3–6 cm. high; stem not tinged olive.

arenaria. Plant 2–4 cm. high; stem squamulose, tinged olive.

partita. Ascigerous portion tongue-shaped, more or less divided.

Mitrula purpurascens, *Massee.* (Pl. XII, Fig. 27.)

Caespitose or gregarious, entire fungus 3–6 cm. high, dry, every part blackish or blackish-brown, with a distinct reddish-purple sheen, becoming almost black when dry, imperfectly hollow, flesh dingy purple; ascigerous portion glabrous, variable in form, cylindric-ovate, clavate, or irregularly lobed and deformed, often laterally compressed, 1–2 cm. long, up to 1 cm. broad, often much smaller; stem subcylindrical, minutely squamulose. Asci clavate, apex narrowed, pore blue with iodine, 80–100 × 10 μ; spores 8, irregularly 2-seriate above, 1-seriate below, hyaline, smooth, 3–5-guttulate, and becoming 3-septate, narrowly cylindric-fusoid, ends narrowed, often slightly curved, 24–30 × 5 μ; paraphyses slender, septate, tips clavate, brownish.

Syn.—Geoglossum purpurascens, Persoon, Comm. Fung. Clav. 39, 1797.

Geoglossum atropurpureum, Pers., Obs. Myc. ii, 62, t. 3, f. 5, 1799; Fries, Syst. Myc. i, 490, 1821; Cke., Mycogr. 10, f. 16, 1879.

Clavaria mitrata, Holmsk., Fung. Dan. i, 21, with an excellent fig., 1799.

Mitrula glabra, Karst., Myc. Fenn. i, 30, 1871.

Microglossum atropurpureum, Karst., Rev. Mon. 110, 1885; Sacc., Syll. viii, n. 130, 1889; Rehm, Kr.-Fl. n. 5867, 1896.

Geoglossum Hookeri, Cooke, Hedw. 1875, 9; Cke., Mycogr. 10, f. 15, 1875.

Microglossum Hookeri, Sacc., Syll. viii, n. 128, 1889.

Exs.—*Karsten*, Fung. Fenn. n. 448.

Hab.—On the ground among grass, &c.

Distr.—Britain, France, Germany, Sweden, Finland, Denmark, Russia.

Stem fibrillose or squamulose, firm, paler, club sometimes terete, sometimes compressed or bifid, and, especially in late autumn, ventricose and deformed, distinct from the stem (Fries).

Most authors hitherto have considered the fungus described above as being the species called *Clavaria atropurpurea*, Batsch (Elench. Fung. pp. 133 and 179, tab. xi, f. 47, 1783). There is a general superficial resemblance certainly to the single specimen figured by Batsch, and this resemblance has been accepted as sufficient evidence; whereas, if the authors had taken the trouble to read Batsch's diagnosis of his species, they would have found that he says, 'substantia lignosa-suberosa, alba'; a statement which does not at all agree with the structure of the fungus under consideration. Furthermore, Batsch cites *Micheli*, pl. lxxxvi (obviously a slip for pl. lxxxvii), fig. 2, which represents *Clavaria pistillaris*. Finally, although Batsch's figure shows a purple tinge (in some copies), it is described as 'fulvo-atra.' In all probability Batsch's figure represents some *Clavaria* near to, if not identical with, *C. pistillaris*. On the other hand, the fungus described above is certainly *Geoglossum purpurascens*, Pers. (Comm. Fung. Clav. 39, 1797), and the above diagnosis is drawn up from specimens thus named by Persoon himself, and now in the Kew Herbarium.

The species called *Geoglossum Hookeri*, by Cooke, was founded on a single specimen without locality in the Hookerian Herbarium at Kew, and was described as being black, which is true of the dry state. The structure is identical with that of *Mitrula purpurascens*, which is also black when dry. Cooke was mistaken in describing his *G. Hookeri* as having two kinds of paraphyses; the presumed large ones being simply immature asci, which show clearly the apical pore blue when treated with iodine.

Mitrula Berterii, *Montag.*, Ann. Sci. Nat. sér. ii, t. 3, 351, 1835; *Mont.*, in Flor. Chil. (Crypt.) 397, Atlas, pl. 8, f. 5, 1850; *Sacc.*, Syll. viii, n. 117, 1889; *Cke.*, Mycogr. 104, fig. 180, 1876. (Pl. XII, Figs. 33–35.)

Gregarious, entire fungus 2–4 cm. high; ascigerous portion narrowly cylindrical, both ends slightly narrowed, glabrous, blackish brown with a tinge of purple, 7–14 mm. long, about 2 mm. thick; stem similarly coloured to the fertile portion, and a little thinner, straight or flexuous. Asci narrowly cylindric-clavate, apex slightly narrowed, pore blue with iodine, 70–80 × 5 μ; spores 8, obliquely 1-seriate, or inclined to be 2-seriate upwards, hyaline, continuous, smooth, usually slightly curved, linear-elliptic, 7–10 × 1·5–2 μ; paraphyses slender, tips slightly clavate and tinged with brown or red.

Syn.—Mitrula vinosa, Berk., Flor. Tasm. ii, 273, 1860; Cke., Mycogr. 104, f. 181, 1876; Sacc., Syll. viii, n. 118, 1889.

Hab.—On rotten wood and bark.

Distr.—Juan Fernandez (*Bertero*), Tasmania (*Archer*), New Zealand (*Colenso*, n. 407 *b*).

A portion of the type of *M. Berterii*, collected by Bertero in Juan Fernandez, was sent to Berkeley by Montagne, and is now in the Kew Herbarium; this has been examined, along with Berkeley's type of *M. vinosa*, and the two prove to be identical in every respect.

Mitrula Saccardoa, *Bagnis*, Micol. Romana, in R. Acad. del Lincei, ser. iii, 1, 839, pl. 1, f. 5, 1887; *Sacc.*, Syll. viii, n. 106, 1889.

Ascigerous portion globose-ovoid, stout, apical portion vinous purple, lower part white; stem stuffed, yellowish white. Asci linear-clavate; spores 8, hyaline, tinged vinous, guttulate, asperulate.

Hab.—On fallen and decayed stems and leaves.

Distr.—Italy (outside the gate of St. Pancrazio, Rome, *Bagnis*).

Apparently a very fine and distinct species, but rather imperfectly described. The somewhat coarse figure shows the entire fungus to be 7·5–8 cm. high; ascigerous portion elliptical, 2·5–3 × 1 cm.; stem 5 cm. high, 3 mm. thick at the apex, slightly tapering towards the base, inclined to be flexuous; spores obliquely 1-seriate, 20–24 × 7–9 μ.

Mitrula serpentina, *Massee.* (Pl. XIII, Fig. 68.)

Usually tufted, entire fungus dingy yellowish green or olive-green, stem usually paler, 2–4 cm. high, sometimes much larger; ascigerous portion about half the entire length of the fungus, clavate or subcylindrical, hollow, often compressed, terminating abruptly and irregularly below, glabrous, rather slimy when wet; stem thinner than the ascigerous portion, nearly cylindrical, minutely squamulose or granulose; flesh tinged green. Asci narrowly clavate, apex a little narrowed, pore blue with iodine, 100–130 × 11–12 μ; spores 8, irregularly 2-seriate above, 1-seriate below, hyaline, smooth, narrowly elliptical, ends rather acute, often slightly curved, 3–5-guttulate, then 3-septate, 13–18 × 5 μ; paraphyses slender, septate, straight, apex clavate and tinged green.

Syn.—Clavaria serpentina, O. F. Muell., Zool. Dan. Prod. 256, 1776; Schrank, Baiersche Fl. ii, 571, 1789.

Geoglossum viride, Pers., Comm. 40, 1797; Fries, Syst. Myc. i, 489, 1821; Cke., Mycogr. 9, fig. 14, 1875 (spores too large); Phil., Brit. Disc. 32, 1887.

Clavaria mitrata, β viridis, Holmsk., Fung. Dan. 24, with fig., 1798.

Clavaria viridis, Schrad., Flor. Dan. tab. 1258, f. 1, 1791.

Leotia geoglossoides, Corda, Icon. Fung. iii, 37, fig. 94, 1839.

Leotia viridis, Fuckel, Symb. Myc. 284, 1869–70.

Mitrula viridis, Karst., Myc. Fenn. i, 29, 1871; Massee, Brit. Fung.-Fl. iv, 482, 1895; Sacc., Syll. viii, n. 124, 1889.

Leptoglossum viride, Cke., Mycog. 250, 1879.

Microglossum viride, Gillet, Disc. France, 25, with fig., 1879; Rehm, Kr.-Fl., n. 5866, 1896.

Exs.—Rab., Fung. Eur. n. 524, 1625; *Rehm*, Ascom. 151; *Phil.*, Elv. Brit. 54; *Vize*, Brit. Fung. 482; *Libert*, Crypt. Ard. Fasc. ii, n. 123; *Klotzsch*, Herb. Myc. ed. *Rab.* n. 239; *Fckl.*, Fung. Rhen. n. 1140; *Cke.*, Fung. Brit. ed. ii, n. 395; *Roum.*, Fung. Gall. Exs. n. 2378; *Desm.*, Cr. France, sér. i, n. 425; *Moug. and Nest.*, Stirp. Cr., n. 994; *Kunze*, Fung. Sel. n. 196; *Karsten*, Fung. Fenn. n. 449; *Westend*, Herb. Crypt. Belg. (*Crepin*), n. 863; *Ellis and Everh.*, N. Amer. Fung. ed. ii, n. 2030.

Hab.—On the ground among grass and moss, in damp places.

Distr.—Britain, Portugal, France, Belgium, Denmark, Sweden, Finland, Germany, Austria, Switzerland, Italy, United States (West Chester, Pa., *Everhart*), Sikkim (Sachoong, 8,000 ft., Yeumtong, 1,200 ft., *Dr.* [now *Sir*] *J. D. Hooker*).

Variable to some extent in colour, depending on locality and season, ranging from yellowish green, through verdigris, to dark olive-green, the stem is usually paler in colour than the ascigerous portion. The entire fungus generally becomes blackish green when dry. Usually rather densely clustered, the individual plants slender, 2–3 cm. high, but sometimes much taller and stouter, as figured by Holmskiold.

Specimen from Persoon examined.

Mitrula olivacea, *Sacc.*, Syll. viii, n. 125, 1889; *Massee*, Brit. Fung.-Fl. iv, 483, 1895.

Gregarious or caespitose, entire fungus 3–4 cm. high, dry, dingy olive-green, sometimes with a purple sheen, stem paler, entirely blackish green when dry; ascigerous portion 1–2 cm. long, up to 5 mm. broad, cylindric-ovate, apex usually narrowed, sometimes obovate or irregular in form, glabrous, more or less compressed and grooved; stem subcylindrical, glabrous, base slightly thickened and whitish. Asci narrowly clavate, apex narrowed, pore blue with iodine, 90–100 × 10–11 μ; spores 8, irregularly 2-seriate above, 1-seriate below, narrowly elliptical, ends rounded, 3–4-guttulate, hyaline, smooth, continuous, often slightly curved, 15–19 × 5–6 μ; paraphyses slender, tips slightly clavate and tinged olive.

Syn.—*Geoglossum olivaceum*, Pers., Obs. Myc. i, 40, tab. 5, f. 7, 1796; Cooke, Mycogr. 9, fig. 14, 1875.

Leptoglossum olivaceum, Cke., Mycogr. 250, 1879; Phil., Brit. Disc. 33, 1887.

Microglossum olivaceum, Gillet, Disc. France, 26, with a fig., 1879; Rehm, Kr.-Fl., n. 5868, 1896.

Geoglossum olivaceum, var. *purpureum*, Berk., Outl. pl. 22, f. 2, 1860.

Geoglossum carneum, Schultz, Flor. Starg. 486, 1806.

Exs.—*Phil.*, Elv. Brit. n. 5; *Cke.*, Fung. Brit. n. 650; *Cke.*, Fung. Brit. ed. ii, n. 396; *Jack*, *Leiner*, *u. Sitzenb.*, Krypt. Badens, n. 650; *Rab.*, Fung. Eur. n. 1820; *Kunze*, Fung. Sel. n. 195.

Hab.—On the ground among short grass, moss, &c.

Distr.—Britain, France, Germany, Belgium, Finland.

The β *purpureum* of Berkeley differs only in colour, which is dingy purple when fresh, and becomes indistinguishable from the typical form when dry. The same is true of the form called *Geoglossum carneum*, Schultz, which is said to be slender, compressed, flesh-colour, stem paler. The colour of the type-form, even in the same patches, varies in different individuals to brown, olive, and purple.

Mitrula lutescens, *Massee* (non *Berk.*). (Pl. XIII, Fig. 77.)

Scattered or fasciculate, entire fungus 3–7 cm. high; ascigerous portion 15–20 mm. long, 1–10 mm. broad, at first subcylindrical, then compressed, lanceolate, apex rarely rounded, yellowish olive or greyish ochraceous, solid, flesh pale ochraceous, sharply differentiated from the stem, which is similar in colour except at the base, which is greenish both inside and out, minutely squamulose, especially upwards. Asci inoperculate, subclavate, slightly narrowed towards the base, 130–150 × 15–17 μ; spores 8, irregularly 2-seriate, oblong-fusoid, straight or slightly curved, hyaline, 3–4-guttulate, 20–26 × 6–7 μ; paraphyses slender, irregularly branched from the base, tips clavate or subcapitate, 3–4 μ thick, and often coated with mucilage.

Syn.—*Microglossum lutescens*, Boudier, Bull. Soc. Myc. France, 14, pl. iv, f. 1, 1896.

Hab.—On sandy clay ground, sides of roads in woods.

Distr.—France (Montmorency, *Boudier*).

This species agrees in many respects with *M. viridis*, but I consider it quite distinct by the colour being more ochraceous than green except at the base of the stem, by the ascigerous portion being relatively shorter, more lanceolate, broader in the middle, and more compressed, also by the stem being finely squamulose; the spores are also a little larger and the asci a little smaller. It occurs on ground which is not pure clay. It is also neighbour to *M. olivacea*, but is well distinguished by its more ochraceous colour, general smaller size, stem longer than the ascigerous portion and greenish at the base, paraphyses usually not curved at the tips, and by the slightly larger and straighter spores. During drying, the present species becomes greener, whereas under the same conditions *M. viridis* becomes yellower (Boudier).

Mitrula laricina, *Massee*. (Pl. XIII, Fig. 69.)

Gregarious, entire fungus 2–6 cm. high; ascigerous portion variable in size and shape, clavate, subglobose, or ovate, sometimes more or less divided into two portions, often more or less compressed, especially when large, fragile, glabrous, becoming hollow, entirely adnate to the stem, but the lower margin sharp and distinct, usually slightly decurrent down opposite sides of the stem, varying in colour from egg-yellow to deep orange-red, ½–1½ cm. high and broad; stem 2–5 mm thick, straight or wavy, smooth and with a silky sheen, white, or with a tinge of pink or yellow, becoming hollow. Asci narrowly clavate, apex narrowed, the minute pore slightly blue with iodine, 100–130 × 8–9 μ; spores 8, obliquely 1-seriate or often irregularly 2-seriate near the apex, hyaline, smooth, elliptical ends blunt, usually slightly curved, often becoming distinctly 1-septate at maturity, 12–18 × 3·5–4 μ; paraphyses septate, sometimes branched, slightly thickened at the tip, about 2 μ thick.

Syn.—*Helvella laricina*, Villars, Fl. Dauph. iii, 1045, tab. lvi (quoted lv in text), 1786–89.

Clavaria phalloides, Bull., Champ. France, 214, pl. 465, f. 3, 1791–98.

Clavaria epiphylla, Dickson, Plant. Crypt. iii, 22, tab. ix, f. 10, 1793.

Leotia laricina, Pers., Syn. Fung. 614, 1801.

Leotia Ludwigii, Pers., Syn. Fung. 611, tab. 3, f. 13, 1801.

Leotia Dicksoni, Pers., Syn. Fung. 612, 1801.

Leotia Bulliardi, Pers., Syn. Fung. 612, 1801.

Mitrula paludosa, Fries, Syst. Myc. i, 491, 1821; Phil., Brit. Disc. 28, pl. ii, f. 6, 1887; Cooke, Mycogr. 101, fig. 175, 1879.

Mitrula phalloides, Chev., Fl. Paris, 114, 1826–27; Sacc., Syll. viii, n. 99, 1889; Massee, Brit. Fung.-Fl. iv, 481, figs. 29 and 30 on p. 188, 1895; Rehm, Kr.-Fl. n. 5860, figs. 1–4 on p. 1143, 1896.

Leotia uliginosa, Grev., Scot. Crypt. Fl. tab. 312, 1823.

Mitrula paludosa, var. *pachyceps*, Karst., Revis. Mon. Ascom. Finl. 110, 1885.

Exs.—*Roum.*, Fung. Gall. 3722; Flor. Exs. Austr.-Hung. n. 1975; *Phil.*, Elv. Brit. n. 2; *Rehm*, Ascom. n. 601; *Rabenh.-Winter*, Fung. Eur. n. 2844; *Fckl.*, Fung. Rhen. n. 1236; *Klotzsch*, Herb. Myc. ed. *Rabenh.* n. 132; *Roum.*, Fung. Sel. Gall. n. 160; *Zopf and Sydow*, Myc. March. n. 31, with fig.; *Karst.*, Myc. Fenn. n. 24; *Thümen*, Myc. Univ. n. 111; *Moug. and Nestler*, Stirp. Crypt. n. 685; *Desm.*, Crypt. France, sér. 1, n. 606; *Berk.*, Brit. Fungi, n. 278; Flor. Gall. and Germ. Exs. n. 796; *Rav.*, Fung. Carol. Exs. n. 36; *Ellis*, N. Amer. Fung. n. 433.

Hab.—On decaying leaves and rotten wood in damp places, or when floating in water.

Distr.—Britain, France, Portugal, Italy, Germany, Sweden, Denmark, Belgium, Switzerland, Finland, Russia, United States.

When exceptionally large and having the pileus laterally compressed, and with well-developed, decurrent lobes on opposite sides of the stem, the present species superficially resembles *Spathularia clavata*, from which species it is readily distinguished by the very different spores. Bulliard's specific name is antedated by that of Villars, as given above; the date given by Saccardo for Bulliard's work is that of the last-named author's Hist. des Pl. Vén. et suspectes de la France, and not the Hist. des Champ. which forms the second part of the Herb. de la France, dating 1791–98.

Form **alba**.

Entirely white, head subglobose; otherwise as in the typical form; spores often 1-septate.

Syn.—*Mitrula alba*, W. G. Smith, Grev. i, 136, pl. 10, lower fig., 1872; Cooke, Mycogr. 102, f. 177, 1875; Phil., Brit. Disc. 27, 1887; Sacc., Syll. viii, n. 103, 1889.

Mitrula phalloides, v. *alba*, Massee, Brit. Fung.-Fl. iv, 482, 1895.

Hab.—Among damp or submerged dead leaves.

Distr.—England (East Budleigh, *Perceval*; Carlisle, *Dr. Carlyle*), Italy (*Bagnis*).

Mitrula vitellina, *Sacc.*, Misc. Mycol. ii. 15, 1885; *Sacc.*, Syll. viii, n. 115, 1889; *Rehm*, Kr.-Fl. n. 5865, 1896. (Pl. XII, Figs. 3–4*a*.)

Ascigerous portion subcylindrical, terete or somewhat compressed, apex narrowed, glabrous, bright egg-yellow, 1–1·5 cm. long, 2·5–3 mm. thick; stem somewhat compressed, white, fibrillose, stuffed, 1·5 cm. long, 2·5–3 mm. thick, substance spongy-fleshy, white. Asci narrowly clavate, apex rounded or inclined to be truncate, not blue with iodine, 75–80 × 7–8 μ; spores 8, obliquely 1-seriate, or inclined to be 2-seriate above, continuous, smooth, elliptical, often slightly inaequilateral, with a very slight yellow tinge, 7–8 × 4 μ; paraphyses slender, septate, slightly thickened upwards, rare.

Syn.—*Geoglossum* (*Microglossum*) *vitellinum*, Bresadola, Revue Myc. iv, 212, 1882; Bres., Fung. Tridentini, 41, tab. xlv, f. 1, 1881.

Hab.—Alpine pine woods.

Distr.—South Tirol (Valle di Rabbi, *Bresadola*).

Almost similar in habit and colour, except the stem, to *Clavaria ligula*, with which it has probably been confounded. Intermediate between *Geoglossum* and *Mitrula* (Bres.).

Specimens from Bresadola examined.

Mitrula elegans, *Fries*, Symb. Nov. 119, 1851; *Berk.*, Grev. iii, 149, 1875; Hedw. 1875, 9; *Cooke*, Mycogr. 104, fig. 182, 1875; *Sacc.*, Syll. n. 119, 1889.

Gregarious, entire fungus 4–7 cm. high, dingy yellow when dry; ascigerous portion narrowly obovate, laterally compressed and slightly decurrent down opposite sides of the stem, glabrous, even, 4–7 mm. high; stem elongated, slender, almost equal throughout, sometimes forked, glabrous, 3–4 mm. thick. Asci narrowly clavate, apex slightly narrowed, pore blue with iodine, 100 × 8–9 μ; spores 8, 2-seriate above, 1-seriate near base of the ascus, smooth, continuous, linear-fusiform, usually slightly curved, 12–14 × 3 μ; paraphyses slender, septate, very slightly thickened at the tip.

Syn.—*Leotia elegans*, Berk., Lond. Journ. Bot. v, 6, 1846.

Hab.—On the ground.

Distr.—United States (*Greene*, n. 66), Ohio (*Lindblom*).

A striking species, remarkable for the long, slender stem, which at
st s gests having been drawn up by growing amongst long
s, but there are eight specimens in the Berkeley Her-
m at Kew, collected by Greene, and the same form was seen by
must be assumed to be normal. The stem is some-
he colour of the living fungus is not known; it was
y pale yellow. Type examined.

Mitrula luteola, *Ellis*, Amer. Nat. xvii, pt. i, 192, 1883; *Sacc.*, Syll. Suppl. x, n. 4466, 1892. (Pl. XII, Figs. 23, 24 *a*.)

Entire fungus 1·5–3 cm. high; ascigerous portion ovate or obovate, subplicate, usually laterally compressed, slightly decurrent down opposite sides of the stem, light yellow, ½–¾ cm. wide; stem shorter than the fertile portion and paler in colour, distinctly tomentose throughout its length, often slightly hollow. Asci narrowly cylindric-clavate, apex rounded or slightly truncate, not blue with iodine, about 100 × 6 μ; spores 8, obliquely 1-seriate, continuous, smooth, elliptical, often very slightly inaequilateral, with a very faint tinge of yellow in the mass, 6–7 × 3 μ; paraphyses rare, stout, septate.

Exs.—*Ellis*, N. Amer. Fung. n. 978.

Hab.—On the ground among fallen pine leaves in sandy pine woods. Solitary or subcaespitose.

Distr.—United States (Newfield, N.Y., *Ellis*).

A very fine and distinct species, known by the tomentose stem, the narrow asci, and the small spores. The fungus becomes darker when dry, more especially the stem. Specimen from Ellis examined.

Mitrula rufa, *Sacc.*, Syll. viii, n. 123, 1889. (Pl. XII, Figs. 28–30.)

Gregarious or scattered, entire fungus 3–6 cm. high, dry, varying in colour from rufous or dusky brownish olive to dingy yellow, stem usually paler than the remainder; ascigerous portion narrowly elliptical or clavate, often more or less laterally compressed and longitudinally rugulose, glabrous, 1–2 cm. long, 4–7 mm. broad, not sharply differentiated from the thinner, minutely squamulose stem. Asci clavate, apex narrowed, pore blue with iodine, 115–140 × 10–12 μ; spores 8, irregularly 2-seriate near the apex of the ascus, 1-seriate below, hyaline, smooth, cylindric-fusoid, ends rather acute, usually slightly curved, 25–35 × 5–6 μ, at first multiguttulate, finally 5-septate; paraphyses slender, septate, tip slightly thickened and more or less curved.

Syn.—*Geoglossum rufum*, Schweinitz, Syn. Fung. Amer. Bor. n. 1011, in Trans. Amer. Phil. Soc. iv, 181, 1834; Cooke, Mycogr. 205, fig. 346, 1875.

Mitrula lutea, Mont., Ann. Sci. Nat. ser. 4, iii, 91, 1855; Cke., Mycogr. 103, fig. 179, 1875; Sacc., Syll. viii, n. 120, 1889.

Geoglossum luteum, Peck, 24th Rep. N. Y. State Mus. 94, pl. 3, f. 20–24, 1871; Cke., Mycogr. 8, fig. 12, 1875.

Geoglossum flavum, Cke., Bull. Buff. Soc. Nat. Sci. March, 1875.

Leptoglossum luteum, Sacc., Syll. viii, n. 158, 1889.

Mitrula lutescens, Berk. and Curt., Grev. iii, 149, 1875; Cke., Mycogr. 102, f. 178, 1875; Sacc., Syll. viii, n. 121, 1889.

Geoglossum pistillaris, Berk. and Cooke, Mycogr. 206, fig. 348, 1876.

Mitrula pistillaris, Sacc., Syll. viii. n. 122, 1889.

Exs.—*Ellis*, N. Amer. Fung. n. 58.

Hab. On the ground among damp moss and grass, also in swamps among *Sphagnum*.

Distr. United States (Louisiana, *Dr. Hale*, n. 4825; Lower Carolina, *Ravenel*, n. 2880; Newfield, N.Y., *Ellis*; Sandlake, N.Y., *Peck*; N. Jersey, specimen from Herb. Schweinitz), Guiana (*Leprieur*, n. 1075)

A well-marked fungus, slightly variable in colour, but very constant in all essential features. The amount of scaliness of the stem varies to some extent, being in some specimens very evident, in others rudimentary, but always very slight. The fungus is normally dry, but, as in other species, may become slightly viscid in rainy weather. All Berkeley's types have been examined; a specimen of *Geoglossum rufum*, from Herb. Schweinitz; also specimens of the type of *Mitrula lutea*, collected by Leprieur in Guiana, and sent by Montagne to Berkeley.

Mitrula cucullata, *Fries*, Epicr. 584, 1836–38; *Phil.*, Brit. Disc. 27, 1887; *Sacc.*, Syll. viii, n. 100, 1889; *Massee*, Brit. Fung.-Flora, iv, 482, 1895; *Cke.*, Mycogr. 101, f. 176, 1875; *Rehm*, Kr.-Fl. n. 5861, 1896. (Pl. XII, Figs. 39–41.)

Gregarious; entire fungus 1–2·5 cm. high, dry; ascigerous portion variable in shape, ovate, obovate, or subglobose, usually somewhat laterally compressed, lower margin often free from the stem for a very short distance, hollow, glabrous, even, orange-yellow or orange-brown, 2–4 × 2 mm.; stem very slender, usually crooked, glabrous, brown, base downy and darker in colour. Asci narrowly clavate, apex narrowed, pore blue with iodine, 60–70 × 6 μ; spores 8, irregularly 2-seriate, hyaline, smooth, continuous, narrowly fusiform, straight or slightly curved, 12–18 × 3 μ; paraphyses slender, tips thickened.

Syn.—Elvella cucullata, Batsch, Elench. Fung. 189, fig. 132, 1786.

Mitrula Heyderi, Pers., Tent. Disp. meth. Fung. 56, pl. 3, f. 12, 1797.

Leotia mitrula, Pers., Syn. Fung. 611, 1801; Grev., Scot. Crypt. Fl. tab. 81, 1823.

Clavaria ferruginea, Sow., Eng. Fung. pl. 84, 1797.

Mitrula (*Heyderia*) *Abietis*, Fries, Syst. Myc. i, 492, 1821.

Geoglossum cucullatum, Fries, Elench. Fung. i, 233, 1828.

Verpa ferruginea, Wallr., Flor. Germ. Crypt. ii, 549, 1833.

Exs.—Rab., Fung. Eur. n. 37, 669, 1223, 1315, 1345; *Roum.*, Fung. Gall. Exs. n. 713, 2377; *Phil.*, Elv. Brit. n. 53; *Krieger*, Fung. Sax. 922; *Sydow*, Myc. March. n. 1261; *Cke.*, Fung. Brit. n. 232; *Fuckel*, Fung. Rhen. n. 1237; Schweiz. Krypt. n. 431; *Desm.*, Cr. France, sér. 1, n. 1155; *Karsten*, Fung. Fenn. n. 447; *Berk.*, Brit. Fung. n. 254; *Thüm.*, Fung. Austr. n. 1014; *Holl, Schmidt, and Kunze*, Deutschl. Schwämme, n. xcviii.

Hab.—On fallen leaves of various conifers, &c.

Distr.—Britain, Portugal, Sweden, France, Germany, Denmark, Italy, Russia, United States (Forestburgh, N.Y., *Peck*, n. 320), Tasmania (*Rodway*; on small twigs of *Eucalyptus*).

The spores are distinctly 1-septate in a specimen named by Persoon, and also in British specimens collected at Carlisle by Dr. Carlyle.

Phil., Elv. Brit. n. 2, and *Ellis*, N. Amer. Fung. n. 433, quoted by *Rehm* (Kr.-Fl. n. 5861) under the present species, belong, in both instances, to *Mitrula laricina*. *Mitrula pusilla*, Fr., growing on fallen leaves of *Pinus silvestris*, differs from the present in its slightly smaller asci and spores, and is perhaps only a small form of *M. cucullata*. *Mitrula sphaerocephala*, Bres., also occurring on larch leaves, differs from *M. cucullata* in its much thicker spores.

Mitrula pusilla, *Fries*, Syst. Myc. i, 493, 1821; *Sacc.*, Syll. viii, n. 101, 1889; *Rehm*, Kr.-Fl. n. 5862, 1896; *Boudier*, Bull. Soc. Myc. France, ix, 10, pl. 2, f. 4, 1893. (Pl. XII, Figs. 47–49.)

Gregarious; entire fungus 4–7 mm. high; ascigerous portion clavate or subglobose, glabrous, rusty yellow, ·5–1·5 mm. high, with a slight indication of a lower free margin encircling the stem, which is filiform, usually crooked, yellow, brownish and downy at the base. Asci narrowly clavate, pore blue with iodine, 45–56 x 6 μ; spores 8, 1-seriate, or inclined to be 2-seriate above, hyaline, smooth, continuous, fusiform, ends acute, often slightly bent, 12–15 x 2·5 μ; paraphyses filiform, tips slightly thickened.

Syn.—*Leotia mitrula*, γ *pusilla*, Alb. and Schwz., Consp. Fung. Nisk., 293, 1805.

Leotia pusilla, Nees, Syst. Myc. 173, pl. 17, f. 160, 1817.

Mitrula fusispora, Preuss, Plant. Hoyersw. n. 157; Sacc., Syll. viii, n. 102, 1889.

Exs.—*Krieger*, Fung. Sax. n. 923.

Hab.—On fallen leaves of *Pinus silvestris*.

Distr.—Germany, France.

A minute species, superficially resembling *Mitrula cucullata*; for distinction see note under the last-named species.

Mitrula sphaerocephala, *Bres.*, Fung. Trid. 66, tab. lxxxi, f. 2, 1881; Sacc., Syll. viii, n. 107, 1889.

Ascigerous portion waxy-fleshy, 1 mm. diameter, lurid ochraceous, glabrous, at length pale primrose with the spores; stem stuffed, expanding into the capitulum, to which it is similar in colour, as is also the flesh, base brown, 4–5 mm. long by $\frac{2}{3}$ mm. thick. Asci cylindric-clavate, the minute pore blue with iodine, 130–150 × 8–12 μ; spores subcylindrical, ends rounded, tinged yellow, minutely granular, 2-seriate, 15–18 × 6–7 μ; paraphyses clavate at the tips, septate, 3–4 μ thick, containing ochraceous granules.

Hab.—On fallen larch leaves.

Distr.—N. Italy.

A near ally of *Mitrula cucullata*, Fr., the spherical headed form of which it closely resembles superficially, but is clearly distinct in the very different form and size of the spores. Bresadola states that as far as he has seen, the ascigerous portion is constantly globose. Not examined.

Mitrula gracilis, *Karsten*, Revis. Mon. 110, 1885; *Sacc.*, Syll. viii, n. 105, 1889.

Entire fungus 2–2·5 cm. high; ascigerous portion variable in shape, generally oblong or ovoid, obtuse, fragile, glabrous, orange-brown when dry, 1–2 mm. long; stem filiform, equal, flexuous, deep flesh-colour, 2 cm. high. Asci sublanceolate, 60–85 × 5–6 μ; spores somewhat 2-seriate, fusoid, straight, 8–12 × 2 μ; paraphyses filiform.

Hab.—Among dead *Hypnum*.

Distr.—Finland (Knjäscha, on the White Sea, *Karsten*).

Karsten considers the present species as occupying a position intermediate between *Mitrula elegans*, Berk., and *M. phalloides*, Chev. (=*Mitrula laricina*, Mass., of this work).

Mitrula sclerotipes, *Boud.*, Bull. Soc. Bot. France, xxiv, 309, pl. iv, f. 5, 1877 (the specific name was written '*sclerotipus*' by the author); *Cke.*, Mycogr. 220, f. 370, 1879; *Sacc.*, Syll. viii, n. 108, 1889.

Entire fungus rusty yellow, 1·5–2 cm. high, springing from an irregularly elliptical, yellowish sclerotium 8–10 mm. long; ascigerous portion clavate, more or less wrinkled, sharply differentiated from the stem, solid, glabrous, 4–7 mm. high, about 3 mm. thick; stem solid, even, glabrous, slender, sometimes dividing into 2–3 branches, each bearing an ascigerous portion at its apex, whitish and slightly thickened at the point where it springs from the sclerotium. Asci narrowly clavate, apex narrowed, pore blue with iodine, 50 × 4–5 μ; spores 8, irregularly 2-seriate above, 1-seriate below, hyaline, continuous, smooth, narrowly elliptic-fusiform, straight or slightly curved, 7–10 × 2 μ; paraphyses slender, very slightly thickened upwards.

Hab.—On the ground in damp places among moss and dead leaves.

Distr.—France (Montmorency, *Boudier*).

A very distinct species, remarkable for springing from a sclerotium, and also for having the base of the ascigerous portion distinct from the stem for a very short distance, and thus forming a very slight free margin. Specimen from Boudier examined.

Mitrula muscicola, *Henning*, Öfvers. af K. Vet.-Akad. Förh., 1885, 71, tab. viii, f. 6–8; *Sacc.*, Syll. viii, n. 104, 1889. (Pl. XIII, Fig. 73.)

Gregarious; ascigerous portion subglobose-ovoid, rugulose towards the base, ferruginous, stuffed, 8–15 mm. high; stem yellow, flexuous, obsoletely fistulose, 5–12 mm. high, 0·5 mm. thick. Asci cylindrical or clavate, 5 μ thick near the apex; spores 4–6 in an ascus, hyaline, continuous, lanceolate, 9–10 × 2–3 μ; paraphyses filiform, 1·3 μ thick.

Hab.—On *Paludella squarrosa*, Ehrh., and *Racomitrium fasciculare*, Brid.

Distr.—Norway (Hummelfjeld, 1270 metres alt., *Henning*).

Mitrula Rehmii, Bresad., resembles the present species in many points, but is somewhat larger.

Mitrula Rehmii, *Bresad.*, Fung. Trident. ii, 41, tab. cxlvii, f. 2, 1892; *Rehm*, Kr.-Fl. n. 5864, figs. 2–3, p. 1143 (after Bresadola); *Sacc.*, Syll. xi, n. 2405, 1895.

Gregarious, entire fungus 2–3·5 cm. high; ascigerous portion obovate, rarely subglobose, stuffed, surface rugose, with thick longitudinal ribs, tawny flesh-colour, 3·5–4·5 mm. high, by about 3 mm. broad; stem stuffed, equal, pruinose then glabrous, white then yellow, 2–3 cm. long, 1 mm. thick; flesh yellow, inodorous. Asci clavate-fusoid, apex narrowed, the minute pore blue with iodine, 70–80 × 6–8 μ; spores narrowly elliptical, hyaline, smooth, usually slightly curved, at length 1-septate; paraphyses filiform, branched, containing ochraceous granules, tips slightly thickened, about 2 μ thick.

Hab.—On moss, *Hypnum Schreberi*, in pine woods.

Distr. South Tyrol (Valle di Sole, *Bresadola*).

Bresadola says that this species is parasitic on the moss. Allied to *Mitrula muscicola*, Henning, differing mainly in the somewhat longer stem and more uneven hymenium.

Mitrula bicolor, *Pat.*, Journ. de Bot. vii, 344, 1893; *Sacc.*, Syll. Suppl. xi, n. 2404, 1895.

Solitary, erect, 6 cm. high, quite glabrous; ascigerous portion elongate-lanceolate, 2 cm. long, 4 mm. broad, compressed, here and there plicate, the attenuated apex obtuse, lower part narrowed into the stem, brown; stem subterate, channelled, 2 mm. thick, flexuous, rigid, pellucid, fuscous. Asci subclavate, apex narrowed, 110–120 × 12–15 μ; spores 8, oblong, ends obtuse, straight or slightly curved, hyaline, continuous, with 1–2 large oil-globules, 10–12 × 6 μ; paraphyses filiform, tips slightly thickened.

Hab.— (?)

Distr.—Thibet (province of Su-tchuen, *Farges*).

This species is the size of *Mitrula rufa*; the ascigerous portion and summit of the stem are reddish brown with a green tinge (Patouillard).

Mitrula multiformis, *Massee.*

Gregarious; ascigerous portion fuscous-brown, glabrous, even or rugulose, distinct from the stem, usually clavate, compressed, hollow; rarely capitate, subrotund, hollow; or pileate, campanulate, undersurface sterile, plane, whitish, stuffed; stem whitish, even, equal or slightly thickened downwards, fistulose, 1·5 mm. thick. Asci cylindric-clavate; spores 8, continuous, hyaline, fusoid, rarely subglobose; paraphyses filiform, septate (?).

Form **clavata**. (Pl. XIII, Fig. 58.)

Ascigerous portion 5–18 mm. high, 3–6 mm. broad; stem 1–2 cm. high. Asci 6–8 μ thick near the apex, 3–4 μ thick at the base; spores 9–13 × 3·5–4 μ; paraphyses 1·5 μ thick.

Form **capitata**. (Pl. XIII, Fig. 59.)

About 1 cm. high; asci 7 μ thick near the apex, base 3 μ thick, or cylindrical and 5 μ thick. Spores 11–12 × 3–4 μ; paraphyses 1·5 μ thick.

Form **pileata**. (Pl. XIII, Fig. 60.)

About 1 cm. high; apex of ascus 6–7 μ broad, base 2·5–4 μ;

spores 7–9 and 2·5–3·5 μ, or subglobose and 3–5 μ diameter; paraphyses 1·3 μ thick.

Syn.—*Geoglossum multiforme*, Henn., Öfvers. af K. Vet.-Akad. Förh., 1885, 70, tab. viii, f. 1–5.

Microglossum multiforme, Sacc., Syll. viii, n. 129, 1889.

Hab.—In swampy places. Forms *capitata* and *clavata* on *Hypnum fluitans*, L., form *pileata* on fallen twigs and leaves of *Betula*.

Distr.—Norway (Hummelfjeld, *Henning*).

Mitrula globosa, *Sommerf.*, Suppl. Flor. Lapp. 287, pl. iii, 1826; *Fries*, Elench. i, 224, 1828; *Sacc.*, Syll. viii, n. 109, 1889. (Pl. XIII, Fig. 54.)

Scattered; entire fungus 3–5 cm. high, every part dark bay; ascigerous portion globose, even, solid, about 5 mm. diameter, not decurrent down the stem, which is about 2 mm. thick, narrowed towards the base, slightly crooked, solid, glabrous.

Hab.—Sandy ground.

Distr.—Lapland (Saltdalen, Nordland, *Sommerfelt*).

The present species, although imperfectly described, appears to be a very marked fungus, and should be found again. Sommerfelt states that he observed it for several years in the same locality, in October. Sommerfelt's figure is reproduced.

Mitrula sclerotiorum, *Rostr.*, Mykol. Meddel. 1888, p. 10; Sacc., Syll. viii, n. 113, 1889.

Ascigerous portion globose-ellipsoid, pallid, margin adnate to the stem, which is 5–8 mm. long, ½–1 mm. thick. Asci cylindrical, pedicel elongated, 35–60 × 4–5 μ; spores fusoid, 7–8 × 1–2 μ.

Hab.—Springing from a sclerotium on the fallen stems of *Lotus* and *Medicago*.

Distr.—Germany (Constanz, *Rostrup*).

May possibly prove to belong to some genus belonging to the Pezizeae.

Mitrula microspora, *Massee*, Brit. Fung.-Fl. iv, 483, 1895. (Pl. XIII, Figs. 55–57.)

Entire fungus 3–6 cm. high, black; ascigerous portion narrowly clavate, obtuse, sometimes irregular in form and more or less laterally compressed, glabrous, differentiated from the stem and a little shorter, 1·5–2 cm. long, 4–7 mm. broad, more or less viscid when moist;

stem cylindrical, mostly equal throughout, minutely squamulose under a lens, or in some instances almost glabrous. Asci narrowly clavate, apex narrowed, pore blue with iodine, 115–130 × 12 μ; spores irregularly 2-seriate above, 1-seriate below, hyaline, smooth, narrowly cylindrical, ends slightly narrowed, multiguttulate, then becoming 3–5–7-septate, usually slightly curved, 28–40 × 5 μ; paraphyses numerous, cylindrical, septate, not at all or very slightly thickened at the tips, which are agglutinated together.

Syn.—Geoglossum microsporum, Cke. and Peck, 25th Rep. N.Y. State Mus. 97, 1872; Cke., Mycogr. 1, f. 11, 1875 (spore-measurement wrong); Phil., Brit. Disc. 39, 1887 (spore-measurement wrong).

Leptoglossum microsporum, Sacc., Syll. viii, n. 157, 1889.

Exs.—Desm., Crypt. France, sér. 1, n. 421 (called *Geoglossum glabrum*, Pers.).

Hab.—On the ground, under ferns, &c.

Distr.—Britain, France (*Desm.*, Crypt. France, n. 421, called *Geoglossum glabrum*, P.), United States (Greig, N.Y., *Peck*, n. 115).

The above diagnosis is drawn up from the type specimen sent from the United States by Peck, and on which the species is founded. In the original description in the 25th Rep. N. Y. State Mus. p. 97, the spore measurements are ·0007′–·0013′ long.

Some time afterwards Cooke again published the same species in Mycographia 8, f. 11, as *Geoglossum microsporum*, C. and P., adding, 'Figured from specimens communicated by C. H. Peck.' I have examined every specimen sent to Cooke by Peck from America, but find nothing agreeing with Cooke's measurements, which I imagine to represent an uncorrected mistake, as the figures of the spores given by Cooke do not justify the statement. Phillips, in Brit. Disc., p. 39, has unfortunately given Cooke's incorrect diagnosis from Mycographia instead of the approximately correct one from the 25th Report. It is evident that Phillips had not examined the specimens; finally, if Mr. C. Bucknall's specimen found at Hanham, Clifton, has spores 10 μ thick, it is not *G. microsporum*, but a new species which would naturally be called *Mitrula microspora*, Massee.

Var. **tremellosa**, *Mass.*, Brit. Fung.-Fl. iv, 484, 1895.

Somewhat tremelloid when living; ascigerous portion subcompressed, hollow, stem smooth, in other respects conforming with the typical form.

Syn.—Geoglossum microsporum, C. and P., var. *tremellosum*, Cke., Grev. iv, 109, 1876.

Geoglossum tremellosum, Cke., Mycogr. 206, f. 347, 1879; Phil., Brit. Disc. 39, 1887.

Leptoglossum tremellosum, Cke., Syll. viii, n. 156, 1889.

Hab.—On the ground.

Distr.—Scotland (Rannoch, *Dr. Buchanan White*).

As shown by the synonymy, this was first considered as a variety of *M. microspora*, and afterwards raised to specific rank. I prefer the varietal position, and indeed have doubts as to whether it is more than a mere form.

Var. littorale.

Scattered or caespitose, 0·5–1·5 cm. broad, black; ascigerous portion clavate, unequal, compressed and wrinkled, brittle, viscid. Asci fusiform, 100–120 × 16–18 μ; spores cylindrical, hyaline, 1–5, usually 2-septate, 50–60 × 5 μ; paraphyses septate, brown, constricted.

Syn.—Leptoglossum littorale, Rostrup, Bot. Tidssk. xviii, 76, 1892; Sacc., Syll. Suppl. xi, 2408, 1895.

Hab.—Sandy ground.

Distr.—Denmark (*Rostrup*).

Unknown to me. Judging from the diagnosis, this species will possibly prove to be only a form of *M. microspora*.

Mitrula arenaria, *Massee*.

Scattered or caespitose, 2–4 cm. high, 0·5–2 cm. thick, black; ascigerous portion irregularly clavate, compressed, even; stem squamulose, tinged olive. Asci fusiform, narrowed below into a long pedicel, 100 × 12–13 μ; spores elongato-cylindrical, hyaline, continuous, often with several oil-globules, 25–30 × 4–6 μ; paraphyses brown, septate, tips slightly clavate, crooked.

Syn.—*Microglossum arenarium*, Rostrup, Bot. Tidssk. xviii, 76, 1892; Sacc., Syll. Suppl. xi, n. 2406, 1895.

Hab. Sandy ground.

Distr.—Denmark (*Rostrup*).

Unknown to me. Appears to differ from *M. microspora* in the continuous spores and the olive stem.

Mitrula partita, *Massee*.

Glabrous, black, coriaceous, gelatinous; ascigerous portion tongue-shaped, usually split down its entire length, and sometimes each portion is again incised to a greater or less depth, hymenium covering every part, 15 mm. long by 6–8 mm. broad; stem glabrous, slender, 2–5 cm. long, 3 mm. broad. Asci elongated, obtuse, becoming blue with iodine round the pore, 130 × 10 μ; spores 8, somewhat 2-seriate, hyaline, continuous, straight, curved, or undulate, elongate-fusoid, 20–30 × 3–6 μ; paraphyses linear, hyaline; the tips of the paraphyses and asci encrusted with a brown substance.

Syn.—*Microglossum partitum*, Pat., Rev. Mycol., 1890, 135, pl. cvii *bis*, fig. 2; Sacc., Syll. Suppl. x, n. 4467, 1892.

Hab.—On the ground.

Distr.—China (Tsang-chan, above Ta-li; *M. l'abbé Delavay*).

Patouillard considers the present species as allied to *Microglossum Hookeri* (=*Mitrula purpurascens* of this work); but quite distinct in the divided fertile portion, and the shape of the paraphyses.

Doubtful species.

Mitrula (?) antarctica, *Speg.*, Fung. Patag. n. 58, in Bol. Acad. Nacional de Cien. de Córdoba, xi, 54, 1887; *Sacc.*, Syll. viii, n. 126, 1889.

Solitary or in groups of 2–3 individuals, entire fungus 4–7 cm. high, quite even and glabrous; ascigerous portion round when young, then laterally compressed, spathulate-clavate, 2–3 cm. long by 6 mm. broad, fistulose, rugulose and plicate in places, white then honey-colour, often waxy looking and pellucid, apex obtuse, rounded, base attenuated and abruptly and irregularly constricted where it joins the stem, which is round, elongated, gradually and slightly thickened upwards, base elongated, attenuated, often contorted, brownish-rufous or brownish flesh-colour.

Hab.—Among rotten fallen leaves in woods.

Distr.—Patagonia (Voces Bay, *Spegazzini*), Fuegia (Ushuvaia, *Spegazzini*).

The above is Spegazzini's description, who states that it was drawn up from living specimens, which were afterwards lost; hence the fructification is unknown.

Mitrula alba, *Massee.*

Entire fungus about 2·5 cm. high, fasciculate or solitary; ascigerous portion cylindrical, even, white, confluent with the stem, which is abruptly black, passing into greenish white; spores $\frac{3}{80}$ of an inch.

Syn.—Geoglossum album, Johnson, Minnes. Myc. n. 630, 1878.
Mitrula Johnsoni, Sacc., Syll. viii, n. 116, 1889.

Hab.—On the ground and on fallen wood in woods.

Distr.—United States (Minnesota, *Johnson*).

Apparently a distinct species, but imperfectly described. Spores measuring $\frac{3}{80}$ of an inch is evidently a slip; but what was intended? Saccardo suggests $\frac{3}{8000}$ (=9–10 μ), but this is not certain. Saccardo's name was created on account of a previous *Mitrula alba*, W. G. Smith; the latter, however, being simply a white form of *Mitrula phalloides*, Chev., Johnson's original specific name has been restored.

Mitrula exigua, *Fries*, Elench. i, 235, 1822; *Sacc.*, Syll. viii, n. 110, 1889.

Minute, simple, scattered; pileus mitriform, shining white; stem semipellucid, base black.

Syn.—Leotia exigua, Schwz., Syn. Fung. Car. Sup. n. 1119, in Soc. Nat. Cur. Lips. 1822, p. 87.

Hab.—Not uncommon on fallen stems. Pileus 4–5 mm. high, inflated, white; stem 2 mm. high (Schwz.).

Distr.—United States (N. Carolina, *Schweinitz*).

LEOTIA, *Hill*.

More or less gelatinous; ascigerous portion pileate, rather fleshy, imperfectly hollow, margin thick and incurved towards the stem, upper surface fertile, under surface sterile; stem erect, central. Asci narrowly clavate, apex more or less narrowed, pore not blue with iodine; spores hyaline, narrowly elliptical, becoming transversely septate, 2-seriate in the ascus; paraphyses present.

Leotia, Hill, Hist. Pl. 43, 1751.

Helvella, Linn., Sp. Plant. ed. iii, 1649, 1764, in part.

Helotium, Fries, Summa Veg. Scand. 354, 1846, in part.

Cudonia, Fries, Summa Veg. Scand. 348, 1846, in part.

Cudoniella, Sacc., Consp. Gen. Disc., in Bot. Central. xxxviii, 1884, in part.

Elvella, *Clavaria*, *Tremella*, *Peziza*, *Phallus*, in part, of old authors.

The present genus resembles *Vibrissea* in the somewhat fleshy pileus having the free, thick margin incurved towards the stem, but differs materially in the much shorter, narrowly elliptical spores which are arranged in a 2-seriate manner in the ascus. Saccardo has placed the present genus in the Bulgarieae on account of its subgelatinous consistency, but I consider its true affinity is with the present group. *Leotia* approaches very closely to *Helotium*; the latter differs in the firmer texture, and in the more or less distinctly parenchymatous cortex and excipulum. This line of demarcation places *Leotia acicularis*, Pers., in *Helotium*.

Distr.—Of the five well-defined species, four are confined to Europe, and all are rare. The fifth, *L. lubrica*, has a wide range—Europe, Sikkim (8–9000 ft.), Tasmania, New Zealand, Victoria, and United States. The doubtful species, if verified, extend the range of the genus to Greenland on the one hand, and South Africa on the other.

KEY TO THE SPECIES.

I. *Pileus more or less tinged green.*

atrovirens. Plant 1–2 cm. high, entirely dark green; stem minutely squamulose.

lubrica. Plant 4–8 cm. high; pileus yellowish-green to dark green; stem not squamulose.

II. *Pileus not green.*

marcida. Plant 4–10 cm. high, every part dingy yellow; stem long, slender, wavy.

aquatica. Plant 1–2 cm. high, entirely whitish; pileus becoming brownish.

stagnalis. Plant 1–2 cm. high; pileus yellowish flesh-colour.

Leotia atrovirens, *Pers.*, Myc. Eur. 202, tab. ix, f. 1–3, 1823; *Fries*, Syst. Myc. ii, 30, 1823; *Sacc.*, Syll. viii, n. 2512, 1889; *Quélet*, Enchirid. 267, 1886; *Rehm*, Kr.-Fl. n. 5881, 1896; *Cke.*, Mycogr. 219, f. 368, 1879 (scales on stem too large). (Pl. XIII, Figs. 81, 82.)

Gregarious, or often in clusters of 2–4 plants, entire fungus 1–2 cm. high; ascigerous portion fleshy, undulate or almost plane, margin thick, incurved, glabrous, blackish-green, 4–8 mm. broad; stem 1–1·5 cm. long, about 3 mm. thick, more or less distinctly squamulose, equal or thickened upwards, coloured like the fertile portion, or paler; flesh pale greenish-yellow. Asci clavate, apex narrowed, pore not blue with iodine, 130–150 × 11–12 μ; spores 8, irregularly 2-seriate above, 1-seriate below, hyaline, smooth, narrowly elliptical, ends obtuse, usually straight, 18–22 × 5–6 μ, 3–5-guttulate; paraphyses slender, septate, often branched, tips scarcely thickened, greenish.

Syn.—*Helotium atrovirens*, Sprengel, Syst. iv, 489, 1827.

Exs.—*Rab.*, Fung. Eur. n. 522 (poor specimens).

Hab.—On the ground in woods, in damp places.

Distr.—Germany, France (Montmorency, *Boudier*).

Requires to be carefully distinguished from dwarf forms of *Leotia lubrica*, Pers. The chief distinctions are, the absence of the piriform tips of the paraphyses, so characteristic of all forms of *L. lubrica*; the somewhat smaller asci, and the more distinctly squamulose stem.

There is a specimen in the Kew Herbarium labelled 'Leotia atrovirens, W. G. Farlow, Mass., U.S.A., n. 4.' This specimen is in reality *L. lubrica*, f. *chlorocephala*, as defined in the present work; therefore if the evidence of *L. atrovirens* occurring in the United States happens to depend on this specimen, it should be cancelled.

Leotia lubrica, *Pers.*, Comm. Fung. Clav. 31, 1797; *Pers.*, Syn. Fung. 613, 1801; *Fries*, Syst. Myc. ii, 29, 1823; *Wallr.*, Flor. Crypt. Germ. iv, 551, n. 2788, also vars. *lacunosa*, *umbonata*, *laevis*, *revoluta*, on same page, 1833; *Berk.*, Outl. pl. 22, f. 1, 1860; *Cke.*, Mycogr. 97, fig. 171, 1875; *Phil.*, Brit. Disc. 22, 1887; *Massee*, Brit. Fung.-Fl. iv, 471, 1895. *Sacc.*, Syll. viii, n. 2510, 1889. (Pl. XIII, Figs. 61–64.)

Gregarious, or in small clusters, somewhat gelatinous, entire fungus 3–9 cm. long; ascigerous portion irregularly hemispherical, inflated, sometimes depressed above, at others more or less umbonate, wavy, margin obtuse, incurved, glutinous, glabrous, varying from dingy yellow with a tinge of green, dingy ochraceous green, to lurid verdigris-green, 1–2 cm. across; stem 3–8 cm. high, 4–8 μ thick, nearly equal, or more or less inflated at the base, pulpy within then hollow, externally yellowish, or tinged green, with very minute, white innate granules. Asci clavate, apex slightly narrowed, pore not blue with iodine, 150–200 × 10–12 μ; spores 8, irregularly 2-seriate above, 1-seriate near the base of the ascus, hyaline, smooth, narrowly elliptical, ends rather pointed, straight or slightly curved, 3–6-guttulate, eventually 5-septate, 19–27 × 5–6 μ; paraphyses very slender, septate, frequently branched, tips piriform and greenish.

Syn.—*Fungus gelatinosus flavus*, Vaill. Paris, 201, tab. ii, f. 7–9, 1727; Müll., Flor. Dan. tab. 719, 1797.

Leotia gelatinosa capitulo subviridi, Hill, Hist. Pl. 43, 1751.

Elvella lubrica, Scop., Plant. Carn. ii, 477, 1772.

Helvella lutea, Berg., Phyt. i, tab. 151, 1783–84.

Peziza cornucopiae, Hoffm., Veg. Crypt. Germ. ii, 26, tab. 6, f. 1, 1790.

Helvella gelatinosa, Bull., Champ. France, 296, tab. 473, f. 2, 1791; Rehm, Kr.-Fl. n. 5880, f. 1–4, p. 1161, 1896.

Leotia lubrica, Pers., Comm. Fung. Clav. 31, 1797; Pers., Syn. Fung. 613, 1801.

Clavaria tremula, Holmsk., Fung. Dan., 27, with an excellent fig., 1799.

Merulius lubricus, Schum., Pl. Saell. ii, 368, 1803.

Hygromitra tremula, Nees, Syst. fig. 144, 1817.

Helvella flavo-virens, Nees, Syst. fig. 162, 1817.

Exs.—*Cooke*, Fung. Brit. n. 231; *Roum.*, Fung. Gall. n. 712; *Rabenh.*, Fung. Eur. n. 714, 2405, 2509; *Phil.*, Elv. Brit. n. 136; *Rehm*, Ascom. n. 101; *Fuckel*, Fung. Rhen. n. 1138; *Sydow*, Myc. March. nn. 278, 667; *Klotzsch-Rabenh.*, Herb. Myc. 29; *Vize*, Brit. Fung. n. 480; *Oudemans*, Fung. Neerl. n. 286; *Karsten*, Fung. Fenn. n. 461; *Thümen*, Fung. Austr. n. 517; *Ellis*, N. Amer. Fung. n. 57; *Thümen*, Myc. Univ. n. 1112; *Berk.*, Brit. Fung. n. 255; *Desmazières*, Crypt. France, sér. i, n. 426; *Westendorp*, Crypt. Belg. n. 763; *Mougeot and Nestler*, Stirp. Crypt. n. 583; *Holl, Schmidt, and Kunze*, Deutschl. Schw. n. ccxxiv.

Hab.—On damp ground in woods and shady places, also in swamps, peat-bogs, &c.

Distr.—Britain, Ireland, Spain, Portugal, France, Switzerland, Belgium, Germany, Sweden, Italy, Finland, Sikkim (Sinchul, 8,600 ft., *Dr.* [now *Sir*] *J. D. Hooker*, n. 131), Tasmania (*Archer*), New Zealand (*Colenso*), Victoria (*Müeller*), United States (Salem, *Schweinitz*; North and South Carolina, *Curtis*, nn. 508, 2400).

A very variable species, both in form and colour, depending upon the amount of moisture and shade to which it is exposed during development. In some cases a dingy ochraceous yellow, with only a tinge of green, is the predominating colour, in other examples the lurid green colour predominates throughout. The commonest European form has the ascigerous portion dingy olive-green, and the stem yellow, with minute, innate squamules or granules. The slimy cap is sometimes almost even, at others much waved, especially at the margin, imperfectly hollow, and either inflated or finally collapsed, sometimes brownish-red. Stem sometimes short and narrowed towards the base, at others elongated and tapering upwards.

It must be clearly understood that the following 'forms,' although apparently distinct when met with illustrating the extreme departure from the typical state, are in reality nothing more than phases of development of the present species in passing from north to south. The small, clustered form—*Stevensoni*, Berk.—is the most northern condition, of which there are specimens in the Kew Herbarium from Sweden (*Fries*), Finland (*Karsten*), Scotland (*Stevenson*), and New Hampshire, U.S. Then follows the typical form; further south we get *chlorocephala*, Sz., and finally passing into the warm subtropical region, we have *stipitata* (Bosc), (= *viscosa*, Fries), the largest and darkest coloured condition of the species. In Europe what I have considered the typical form is most abundant, the 'forms' being rarer, although all are present; whereas in the United States the opposite conditions hold good, so far as is at present known, the three dark-green forms being not uncommon, whereas there are but few records of the typical form.

Form **Stevensoni**.

Usually densely tufted, small, entire fungus 2–3 cm. high; ascigerous portion dingy, dark green; stem paler. Asci, spores, and paraphyses as in typical form.

Syn. *Leotia Stevensoni*, Berk. and Broome, Ann. Nat. Hist., 1879, p. 212.

Leotia chlorocephala, forma *Stevensoni*, Phil., Brit. Disc. 24, 1887; Sacc., Syll. viii, n. 2511, 1889; Massee, Brit. Fung.-Fl. iv, 472, 1895.

Hab. On the ground in woods and shady places, mostly in northern countries.

Distr.—Scotland (Glamis, *Stevenson*), Sweden (Upsala, *Fries*), Finland (*Karsten*), United States (New Hampshire).

Form chlorocephala.

Usually tufted, entire fungus 4–6 cm., high, every part dark, dingy green. Asci, spores, and paraphyses as in the typical form.

Syn.—*Leotia chlorocephala*, Schwz., Syn. Fung. Carol., in Soc. Nat. Cur. Lips., 1822, p 88 (in extract); Fries, Syst. Myc. ii, 30, 1823; Cooke, Mycogr. 98, fig. 174, 1875; Phil., Brit. Disc. 23, 1887; Sacc., Syll. viii, n. 2511, 1889; Massee, Brit. Fung.-Fl., iv, 471, 1895.

Exs.—*Rav.*, Fung. Carol. iv, n. 22; *Ellis and Everh.*, N. Amer. Fung. ser. ii, n. 2032.

Hab.—On the ground in woods, &c.

Distr.—South of England (New Forest, Hampshire, *Miss Broadwood*; Bournemouth, *Massee*; Surrey), United States (S. Carolina, *Ravenel*, n. 1633; Pennsylvania, *Michener*, n. 3979; Salem, *Schweinitz*; Massachusetts; Jacksonville, Florida, *Calkins*; California, *Harkness*, n. 1216), Guiana (*Leprieur*, n. 1073).

Form stipitata. (Pl. XIII, Fig. 65.)

Scattered or gregarious, entire fungus 5–9 cm. high; ascigerous portion irregularly waved or lobed, dark, lurid green, up to 2·5 cm. broad; stem stout, more or less sulcate or lacunose, yellowish, brownish, or green. Asci, spores, and paraphyses as in the typical form.

Syn.—*Tremella* (*Hygromitra*) *stipitata*, Bosc, Berl. Mag. Nat., 1811, 80, tab. 6, f. 14.

Hygromitra stipitata, Nees, Syst. 1817, 40, tab. xv, f. 144 (after Bosc).

Leotia viscosa, Fries, Syst. Myc. ii, 30, 1823; Cooke, Mycogr. 98, f. 173, 1875; Sacc., Syll. viii, n. 2514, 1889.

Leotia lubrica, var. *viscosa*, Quélet, Enchirid. 266, 1886.

Exs.—*Ravenel*, Fung. Amer. n. 173; *Ellis*, N. Amer. Fung. n. 663.

Hab.—Sandy ground.

Distr.—France (*Quélet*), United States (South Carolina, *Ravenel*, nn. 702, 1784, 2315, 2976; Salem, *Schweinitz*).

The following note accompanies Ravenel's specimens, n. 1784:—

'Pileus convex, undulate, not so viscous as *L. lubrica*; dark green, ½–¾ in. broad. Stem hollow, thick or compressed and sulcate, expanding into pileus, covered with a greenish powder, the cavity filled with a gelatinous matter. Sometimes caespitose, in clusters of 2–3–4; growing in light sand beds, mostly preferring roads but little used, generally only the pileus appearing above ground. Autumn and winter. This is probably the plant which Bosc describes as *Tremella stipitata* (*Leotia viscosa*, Fr.); I think it is a large state of *L. chlorocephala*. Found in Aiken and in the Santee Canal, S. Car.'

When of large size, this form appears to be very distinct, but it passes back by every transition into *chlorocephala*, as observed by Ravenel; and when the stem is yellowish, some forms approach the typical form in general appearance.

The most pronounced features of the species, including its forms, are: very slender, branched paraphyses with small piriform tips, ascus and spore-measurements as given in the diagnosis, and the minutely granulose or squamulose stem, which appears to be pulverulent as seen with the naked eye. When the stem is pale coloured, the granules are whitish; when dark coloured, they are green. See note under *L. atrovirens*.

Specimens of the typical form named by Persoon examined.

Leotia marcida, *Pers.*, Syn. 613, 1801; *Pers.*, Myc. Eur. i, 202, 1822; *Fries*, Syst. Myc. ii, 28, 1823. (Pl. XII, Figs. 18–20.)

Fasciculate or gregarious, somewhat gelatinous when moist; ascigerous portion pileate, convex then becoming irregular, often more or less umbonate, margin incurved, wavy, concave below, thin, yellow, about 1 cm. broad; stem 4–10 cm. long, 2–4 mm. thick, wavy, equal or tapering downwards, coloured like the pileus, every part glabrous. Ascus narrowly clavate, apex narrowed and the wall thickened, not blue with iodine, 140–150 × 11–12 μ; spores 8, 2-seriate above, 1-seriate below, fusiform, ends rather acute, usually slightly curved, 4–6-guttulate, finally 5-septate, 30 × 5 μ; paraphyses slender, very slightly or not at all thickened at the apex, straight.

Syn. *Phallus marcidus*, Müll., Flor. Dan., tab. 654, f. 1, 1775.
Cudonia marcida, Quél., Enchir. 267, 1886.
Cudoniella marcida, Sacc., Syll. viii, n. 132, 1889.
Hab. On the ground among moss, &c.
Distr. France, Denmark.

The above description is drawn up from specimens in the Kew Herbarium, which were collected by Bulliard in the forest of Compiègne, and named by Persoon.

Distinguished from *Leotia lubrica* by the unbranched paraphyses, not being thickened at the apex, and the much more slender build of the ascophore, which is not green.

Very slightly subtremellose, not greenish yellow. Agreeing admirably with the figure in Fl. Dan., tab. 654, except that I have not seen the stem more than 2 inches long. The name is also excellent, the whole substance dry, resembling leather; tomentose but not veined on the under surface (Wahlenb.).

Gregarious, slender, slightly gelatinous; not greenish, neither in the specimens I have seen, nor in an unedited figure in the library of Hornemann. Stem 3–4 in. long, 1–2 lines thick, crooked. Pileus ½ in. broad, rarely more, subumbonate, margin inflexed, wavy, concave and same colour underneath (Fries).

Leotia aquatica, *Libert*, in *Roumeg.*, Fung. Sel. Gall. Exs., n. 639 (with diagnosis), 1880; *Pat.*, Tab. Anal. Fung. 32, fig. 75, 1883. (Pl. XII, Figs. 21–22.)

Gregarious; ascigerous portion convex at first, then plane or somewhat depressed, margin slightly incurved, rufous, glabrous, 3–6 mm. broad, rather fleshy; stem slender, 1–2 cm. long, often flexuous, white, cylindrical, glabrous, base tomentose. Ascus clavate, apex slightly narrowed, not blue with iodine, about 70 × 8 μ; spores 8, 2-seriate above, becoming 1-seriate below, elliptical, ends narrowed, sometimes slightly curved, 11–14 × 4 μ; paraphyses slender, slightly clavate and brownish at the tips, more or less agglutinated together.

Syn.—*Cudonia aquatica*, Quélet, Enchirid. 267, 1886.

Cudoniella aquatica, Sacc., Syll. viii, n. 135, 1889; Rehm., Kr.-Fl., n. 5884, 1896.

Hab.—On submerged wood and branches.

Distr.—Germany, France.

Schröter (Krypt.-Flora von Schlesien, iii, pt. ii, p. 21) says the ascigerous portion is 'white when fresh, brownish when dry'; this statement is repeated by Rehm. Both these authors also give the same spore-measurements (7–9 × 2–3 μ), which are less than those found on examining Madame Libert's specimens.

Leotia stagnalis, *Massee.*

Ascigerous portion convex, orbicular, the free margin incurved, glabrous, waxy, rather firm, yellowish flesh-colour, then dingy, 4–5 mm. diameter; stem slender, apex grooved, pale ochraceous, base tinged olive, 1–1·5 cm. long, scarcely 1 mm. thick. Asci (?); spores continuous, narrowly elliptic, 2-guttulate, 12–14 μ long.

Syn.—*Cudonia stagnalis*, Quél., Assoc. Franç. xii, 1883, 13, pl. 7, f. 10.
Cudoniella stagnalis, Sacc., Syll. viii, n. 134, 1889.

Hab.—On leaves and decaying vegetable matter on the margins of swamps.

Distr.—Germany (Alsace, *Quélet*).

Doubtful Species.

Leotia platypoda, *Pers.*, Myc. Eur. i, 202, 1822; *Fries*, Syst. Myc. ii, 28, 1823; *Sacc.*, Syll. viii, n. 2513, 1889.

This species was first described by De Candolle (Flor. Franç. vi, 29, 1815) as follows: ' This species of Helvella is one of the smallest of the genus, and rarely reaches more than six lines high; its consistence is gelatinous, slightly coriaceous; the pedicel is entirely compressed, of a dirty white colour, terminated by an irregularly wrinkled or undulated, small, brownish cap, the margin slightly turned down. M. Aubin found this fungus growing on the ground in the neighbourhood of Grasse, in Provence.'

Syn.—*Helvella platypoda*, De Candolle, Flor. France, vi, 29, 1815.

Hab.—On the ground.

Distr.—France.

The above description does not convey a very clear conception as to what the species was like that De Candolle had in view. Patouillard (Tab. Analyt. Fung. n. 447) had figured and described a fungus which he considers to be the species of De Candolle, collected by MM. Bommer and Rousseau, near Brussels. His description is as follows:—

'Fungus 1-2 cm. high. Pileus gelatinous, wrinkled, undulated, russet. Stem compressed at the summit, attenuated below, whitish. Asci containing eight spores, 2-seriate; spores elongated, clavate, 3–4-guttulate, hyaline; paraphyses branched.'

According to the figure the spores are narrowly cylindric-clavate, 21–22 × 5–6 μ.

Leotia verpoides, *Massee.*

Ascigerous portion discoid, rotund, yellowish, margin entire, recurved, disk dry, wrinkled, even below, pallid, 4 mm. broad; stem 2–3 cm. long, 2 mm. thick, dilated, contorted, brown.

Syn.—*Pezisa verpoides*, Sauter, Hedw., 1876, 149.
Phialea verpoides, Sacc., Syll. viii, n. 1081, 1889.
Cudoniella verpoides, Rehm, Kr.-Fl., n. 5886, 1896.

Hab.—On rotten beech wood.

Distr.—Germany (Salsburg).

This species may possibly prove to belong to *Helotium* rather than to *Leotia.*

Leotia rufa, *Rostrup*, Fung. Groenl. 536, 1888; *Sacc.*, Syll. viii, n. 2517, 1889.

Ascigerous portion wavy, margin revolute, 1–2 mm. broad, rufous; stem unequally terete, rufo-ferruginous, 5–6 mm. high. Asci terete-clavate, pedicellate, 60–70 × 2 μ; spores (?).

Hab.—Among moss.

Distr.—Greenland (Agdluitsok).

Leotia fructigena, *Massee.*

Ascigerous portion hemispherical, ochraceous, 1 mm. diameter; stem 1 cm. high, 0·5 mm. thick, white. Asci 200–220 × 6–7 μ; spores 25–45 × 5 μ, 1–3-septate, hyaline.

Syn.—*Cudoniella fructigena*, Rostrup, Meddel. om Groenland, iii, 591, 1891; Sacc., Syll. Suppl. xi, n. 2407, 1895.

Hab.—On fruits of *Archangelica officinalis.*

Distr.—Greenland.

Judging from the habitat, this species may prove to belong to the genus *Helotium.*

Leotia elegantula, *Kalchbr.*, Grev. x, 143, 1882; *Sacc.*, Syll. viii, n. 2516, 1889.

Subsolitary; ascigerous portion the size of a pea, convex, margin involute, somewhat shining, blackish brown with a purple tinge; rugose and almost lamellose below, ochraceous tan; stem cylindrical, equal, base dilated, pulverulent, ochraceous tan, 2·5 cm. long, 3 mm. thick. Spores shortly ovate, tinged brown, translucent, 9 × 6 μ.

Hab.—(?)

Distr.—S. Africa (Somerset East, *MacOwan*, n. 1310).

Apparently a very remarkable species, respecting which more requires to be known. If the spores are truly coloured, the fungus cannot remain in the present genus, neither does it agree with any other genus at present established.

SPRAGUEOLA, *Massee.*

Ascophore sessile, subglobose, irregularly nodulose, glabrous, solid, hymenium covering the entire surface, attached to the substratum by radiating mycelium. Asci narrowly cylindric-clavate, apex slightly truncate or obtuse, pore blue with iodine; spores 8, obliquely 1-seriate, continuous, hyaline, smooth, elliptical; paraphyses slender, septate.

Spragueola, Massee, Journ. Bot. xxxiv, 144, pl. 357, figs. 8–9, 1896.

Mitrula, Berk., Grev. iii, 149, 1875.

The validity of the present genus is not entirely beyond doubt, partly owing to the fact that, so far as I am aware, only two specimens are known; these are in the Kew Herbarium. Differs from every other genus included in the Geoglosseae in being absolutely sessile, the ascophore being irregularly globose, coarsely nodulose, solid, and everywhere covered by the hymenium. *Mitrula* is the genus most nearly approached in the structure of the asci and spores

Distr.—One species; United States.

Spragueola americana. *Massee*, Journ. Bot. xxxiv, 144, pl. 357, figs 8–9, 1896. (Pl XIII, Figs. 74, 76.)

Ascophore subglobose, attached by a broad base, coarsely nodulose, glabrous, 1·5–2·5 cm. high and broad, pale ochraceous tan (when dry), fleshy, solid, internally white. Asci narrowly cylindric-clavate, apex obtuse, pore blue with iodine, 70–80 × 5–6 µ, spores 8, obliquely 1-seriate, continuous, hyaline, elliptical, smooth, ends obtuse, 6·5–7 × 3·5 µ; paraphyses slender, septate, slightly clavate, straight.

Syn.—*Mitrula crispata*, Fries, Berk., N. Amer. Fung. n. 704*, in Grev. iii, 142, 1875.

Hab. On the ground among pine leaves.

Distr. United States (New England, *Sprague*, n. 5785).

As to what *Spathularia crispata*, Fries, really is, we shall never know, as it has not been described. In first mentioning the name—Summ. Veg. Scand. 347 (1846)—Fries, in contrasting it with *S. flavida*, says, "A priori distinctissima." Fuckel accepts as the species of

Fries a *Spathularia* differing from *S. flavida* in having slightly different spores, measuring 48 × 3 μ, whereas his measurements for *S. flavida* are 72 × 2 μ (Symb. Myc. 332). Berkeley, on the other hand, considered the New England fungus communicated by Sprague to represent *S. crispata* of Fries, but, observing that the spores were elliptical, placed it in the genus *Mitrula*, without, however, giving a diagnosis, but simply stating, 'Sporidia elliptic, uniseriate.' As the fungus under consideration is neither a *Spathularia* nor a *Mitrula*, neither does it accord with any hitherto defined genus, it is named after its discoverer, one of the pioneers of N. American botany (Massee, Journ. Bot. l. c.).

HEMIGLOSSUM, *Pat.*

Ascigerous portion gelatinous, coriaceous, simple or branched, tongue-shaped or convolute, stipitate; hymenium unilateral; asci elongated, opening by a pore; spores 8, small, ovoid or fusoid, hyaline.

Hemiglossum, Patouillard, Rev. Myc. 1890, 135; Sacc., Syl. Suppl. x, p. 2, 1892.

Distinguished at once from all other genera by the more or less incised vertical ascigerous portion having the hymenium developed on one surface only, the other surface remaining sterile. This differentiation of surfaces is also met with in *Leotia* and *Vibrissea*, but in both these genera the ascigerous portion is pileate and horizontal, the upper surface being fertile, the under surface sterile. *Hemiglossum* is analogous in the arrangement of its hymenium with *Skepperia* and *Lachnocladium*, genera belonging to the Basidiomycetes.

Distr.—Only one species known, China.

Hemiglossum Yunnanense, *Pat.*, Rev. Myc. 135, pl. cvii *bis*, fig. 2, 1890; *Sacc.*, Syll. Suppl. x, n. 4468, 1892. (Pl. XIII, Figs. 83, 84.)

Ascigerous portion simple or branched, more or less incised or lobed, margin revolute below or all round, thin, about 1 mm. thick, 10–15 mm. long, 4–8 mm. broad, smooth, tawny; concave sterile face paler, furfuraceous owing to the presence of tufts of ovoid, brown cells; stem rigid, slender, straight or flexuous, reddish brown, 1–2 cm. long, 2–4 mm. thick. Asci stipitate, elongated, apex obtuse, becoming tinged blue round the pore with iodine, 55–60 × 4 μ; spores 8, 1-seriate, hyaline, continuous, fusoid, ends rather acute, 2-guttulate, 6–7 × 1·5–2 μ; paraphyses linear, simple, hyaline.

Hab.—On the ground (?).

Distr.—China (Tsang-chan, above Ta-li, *M. l'abbé Delavay*).

A very remarkable species, the type of structure being unique. I once met with what appeared to be at first sight a structure agreeing exactly with that described above, and was on the point of describing it as a new genus, when I discovered that it was simply a *Mitrula*, the ascigerous portion of which had been crushed and burst open, thus presenting a flattened expansion, more or less incised, and with one surface fertile and the other sterile. I have no evidence that such is the explanation in the present instance.

NEOLECTA, *Speg.*

Ascigerous portion sharply defined, terminal on a stem with which it is homogeneous, terete or spathulato-compressed, bright coloured. Asci cylindrical, 8-spored; spores globose, hyaline, simple; paraphyses absent.

Neolecta, Spegazzini, Fung. Argent., Pug. iv, in Anal. Soc. Cient. Argentina, tom. ix, 83, 1880; Sacc., Syll. viii, 40, 1889.

Agreeing in general habit with the genera *Mitrula* and *Spathularia*, but distinguished from these and every other genus by the globose spores.

Distr. One species, confined to Brazil.

Neolecta flavo-virescens, *Speg.*, Fung. Arg., Pug. iv, Anal. Soc. Cient. Argentina, ix, 83, 1880; Sacc., Syll. viii, n. 131, 1889. (Pl. XIII, Figs. 89, 90.)

Ascigerous portion irregularly cylindrical or clavate-compressed, often spathulate, apex obtuse or wavy and rounded, base more or less distinct, narrowed into the stem, 15–25 mm. high by 2–5 mm. broad; thickish, somewhat elastic and fleshy, even, quite glabrous, rarely here and there grooved and plicate, very bright greenish-yellow; stem subterete, continuous and homogeneous with the fertile portion, even or minutely rugulose, paler, minutely and irregularly rooting below, 4–10 mm. long, 1–1·5 mm. thick. Asci cylindrical or cylindrico-subclavate, apex very obtuse, narrowed below into a long, slender pedicel, 80–85 × 5–6 μ; iodine gives no blue colour, wall of ascus everywhere thin, dehiscing by an operculum; spores 8, globose, hyaline, with 1 large, excentric oil globule, hyaline, 4–5 μ diameter.

Hab.—Among heaps of rotten leaves and twigs.

Distr.—Brazil (Apiahy, *Puiggari*, n. 1544). A fragment of this species was also found among specimens of other fungi sent from Brazil by *Glaziou*.

EXCLUDED SPECIES.

The following are excluded as belonging to genera not included in the Geoglosseae as defined in the present work:—

Vibrissea microscopica, *Berk. and Broome*, Ann. Nat. Hist. ser. 4, xvii, 142, 1876; *Phil.*, Trans. Linn. Soc. (Bot.) ser. 2, ii, 7, pl. 1, f. 17–24, 1881; *Phil.*, Brit. Disc. 319, 1887; *Sacc.*, Syll. viii, n. 175, 1889; *Massee*, Brit. Fung.-Fl. ix, 489, 1895.

This species does not exhibit the characters of *Vibrissea*, and should henceforth be known as *Gorgoniceps microscopica* (B. and Br.).

Vibrissea turbinella, *Sacc.*, Syll. viii, n. 174, 1889.

Syn.—*Peziza turbinella*, Berk., Dec. Fung. n. 358, in Hook., Journ. Bot. iii, 1851.

Peziza stilboidea, Berk., Dec. Fung. n. 359, in Hook., Journ. Bot. iii, 1851.

Vibrissea stilboidea, Sacc., Syll. viii, n. 176, 1889.

The types of both the supposed species have been examined, and prove to be identical. The fungus is a typical *Pocillum*, and should stand as *P. turbinellum* (B.).

Vibrissea pezizoides, *Libert*, ms., in *Phil.*, Gen. Vibrissea, Trans. Linn. Soc. (Bot.) ser. 2, ii, 8, pl. 2, f. 8–13, 1881; *Sacc.*, Syll. viii, n. 172, 1889.

Saccardo has a query as to whether this species is not a *Gorgoniceps*, and on examining the type I find that he was correct; the species will rank as *Gorgoniceps pezizoides* (Lib.).

Vibrissea Guernisaci, *Crouan*, Ann. Sci. Nat. vii, 176, t. 4, f. 24–26, 1857; *Phil.*, Gen. Vibrissea, Trans. Linn. Soc. ser. 2, ii, 8, pl. 2, f. 1–7, 1881; *Phil.*, Brit. Disc. 319, pl. 10, f. 61, 1887; *Massee*, Brit. Fung.-Fl. iv, 488, 1895.

Gorgoniceps Guernisaci, Sacc., Syll. viii, n. 2082, 1889.

Saccardo is correct in placing the present species in the genus *Gorgoniceps*.

Vibrissea leptospora, *Phil.*, Trans. Linn. Soc. (Bot.), ser. 2, ii, 8, pl. 2, f. 19–23, 1881; *Phil.*, Brit. Disc. 320, 1887.

Syn.—*Peziza leptospora*, Berk. & Broome, Ann. Nat. Hist. ser. 3, xviii, 12, tab. 4, f. 30, 1866.

Vibrissea Guernisaci, var. *leptospora*, Massee, Brit. Fung.-Fl. iv, 488, 1895.

Gorgoniceps leptospora, Sacc., Syll. viii, n. 2086, 1889.

Vibrissea Fergussoni, Phil., Trans. Linn. Soc. (Bot.), ser. 2, ii, 7, 1881; Phil., Brit. Disc. 318, 1887; Sacc., Syll. viii, n. 173, 1889.

Patellaria Fergussoni, Berk. & Broome, Ann. Nat. Hist. p. 39, pl. iv, f. 30, 1875.

Gorgoniceps vibrissoides, Sacc., Syll. viii, n. 2084, 1889.

Gorgoniceps turbinata, Sacc., Consp. Gen. Disc. 7.

Helotium vibrissoides, Peck, 32nd Rep. N. Y. State Mus. 48, 1879; Peck, Bull. N. Y. State Mus. 1887, p. 28, pl. 2, f. 7–9.

Vibrissea turbinata, Phillips, Gen. Vibrissea, Trans. Linn. Soc. (Bot.), ser. 2, ii, 8, pl. 2, f. 14–18, 1881.

Vibrissea Guernisaci, var. *vibrissoides*, Massee, Brit. Fung.-Fl. iv, 488, 1895.

Patellaria rosea, Berk. & Curt., in Cooke's Discom. of U. S., pt. ii, in Bull. Buff. Soc. Nat. Sci. 27, 1876; Sacc., Syll. viii, n. 3241, 1889.

In Brit. Fung.-Flora, iv, 488, I have considered *leptospora* and *vibrissoides* as varieties of *Guernisaci*; however, on re-examining a larger series of specimens, I find that the slender varietal characters on which I depended are not constant, and there is no doubt but that *leptospora*, *Fergussoni*, *vibrissoides*, *turbinata*, and *rosea* are identical with, and synonyms of, *Gorgoniceps Guernisaci*, Sacc.

There is a note in Dr. Cooke's handwriting in Herb. Kew. stating that *Helotium vibrissoides*, Peck, is identical with *Patellaria pilifera*, B. & C. I have no knowledge of either specimen or description of the last named species, and I mention this so that, if such a species is extant, its affinities may be known.

Mitrula muscicola, *Fries*, Syst. Myc. i, 492, 1821; Sacc., Syll. viii, n. 111, 1889.

Syn.—*Clavaria muscicola*, Schum. Saell. 405, 1801.

Fries states (Elench. i. 235) that he afterwards saw this species, which proved to be a [illegible].

Mitrula inflata, *Fries*, Elench. i, 234, 1828; *Sacc.*, Syll. viii, n. 112, 1889; *Leotia inflata*, Schweinitz to Fries in litt.

Spathularia inflata, Cke., Mycogr. f. 344, 1879.

This species belongs to the Clavarieae, and is known as *Physalacria inflata*, Peck, Bull. Torr. Bot. Club, 1882, p. 2.

Mitrula minuta, *Fries*, Syst. Myc. i, 492, 1821; *Clavaria minuta*, Sow., Eng. Fung. tab. 391, 1803.

Probably a condition of *Pistillaria micans*, as suggested by Berkeley, Eng. Flora, v, 180.

The following are excluded on account of the impossibility of determining the species intended from the brief diagnoses given; and unless type specimens exist, the names should be no longer allowed to encumber mycological literature.

Leotia unctuosa, *Fries*, Syst. Myc. ii, 31, 1823; *Sacc.*, Syll. viii, n. 2522, 1889; *Rehm*, Kr.-Fl. n. 5882, 1896.

Syn.—*Elvella unctuosa*, Batsch, Elench. Fung. i, 193, f. 134, 1783.

The brief description of five words, even aided by the minute figures, has not enabled any one to identify this species with certainty, hence it always appears in the list of uncertain species.

Leotia amara, *Fries*, Syst. Myc. ii, 27, 1823; *Sacc.*, Syll. viii, n. 2521, 1889.

Syn.—*Helvella amara*, Loureiro, Flor. Cochinch. i, 695, 1790.

Leotia nana, *Fries*, Syst. Myc. ii, 28, 1823; *Phil.*, Brit. Disc. 26, 1887; *Sacc.*, Syll. viii, n. 2522, 1889.

Syn.—*Helvella nana*, Withering, Arr. Brit. Pl., ed. iv, iv, 231, 1801.

Leotia brunneola, *Berk. and Broome*, Fungi of Ceylon, in Journ. Linn. Soc. (Bot.) xiv, 102, 1875; *Sacc.*, Syll. viii, n. 2520, 1889.

Very briefly described from a poor sketch, and no specimens preserved.

Leotia infundibuliformis, *Fries*, Obs. ii, 299, 1818; *Sacc.*, Syll. viii, n. 2519, 1889.

Syn.—*Helvella infundibuliformis*, Schaeff., Fung., tab. 277, 1762; Sowerby, Eng. Fung. pl. 153, 1799.

Leotia atra, *Weinm.*, Syll. Pl. Nov. ii, 109, 1828; *Fries*, Elench. ii, 3, 1828; *Sacc.*, Syll. viii, n. 2518, 1889.

Geoglossum farinaceum, *Schw.*, Fung. Carol. n. 1116, 1822; *Sacc.*, Syll. viii, n. 154, 1889.

The description obviously refers to the conidial condition of some *Hypoxylon*, but specimens alone can settle the species, the diagnosis being too brief.

Geoglossum rugosum. *Lasch*; *Sacc.*, Syll. viii, n. 155, 1889. According to Bot. Ztg., 1846, 45, a specimen bearing this name occurs in Klotzsch-Rabenh., Herb. Myc. n. 816. As a matter of fact, the fungus in question does not occur as indicated, neither can any trace of a specimen bearing the above name be found; and furthermore, as it was never described, the name may be allowed to lapse.

Vibrissea flavipes, *Rabenh.*, in Klotzsch-Rab., Herb. Myc. cent. xvii, n. 27, 1850; Bot. Ztg. 1852, p. 286; *Sacc.*, Syll. viii, n. 171, 1889; *Rehm*, Kr.-Fl. n. 5890, 1896.

This species is synonymous with *Coniocybe nivea*, Rehm.

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EXPLANATION OF FIGURES IN PLATES XII AND XIII.

Illustrating Mr. Massee's Monograph of *Geoglosseae*.

PLATE XII.

Fig. 1. *Geoglossum glabrum*, var. *lignicolum*. Nat. size.
Fig. 2. Spore of same. × 400.
Fig. 3. *Mitrula vitellina*, Sacc. Nat. size (after Bresadola).
Fig. 4. Section of same. Nat. size.
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Fig. 40. Ascus and paraphysis of same. ×400.
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Fig. 47. *Mitrula pusilla*, Fries. Nat. size.
Fig. 48. A single plant of same. Slightly ×.
Fig. 49. Section of same. Slightly ×.

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Fig. 53. Young ascus, paraphyses, and portion of hypothecium of same, showing protoplasmic continuity. ×750.
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Fig. 95. Hair of the same at maturity, springing from an apparently forked base; letters as in Fig. 94. × 400.

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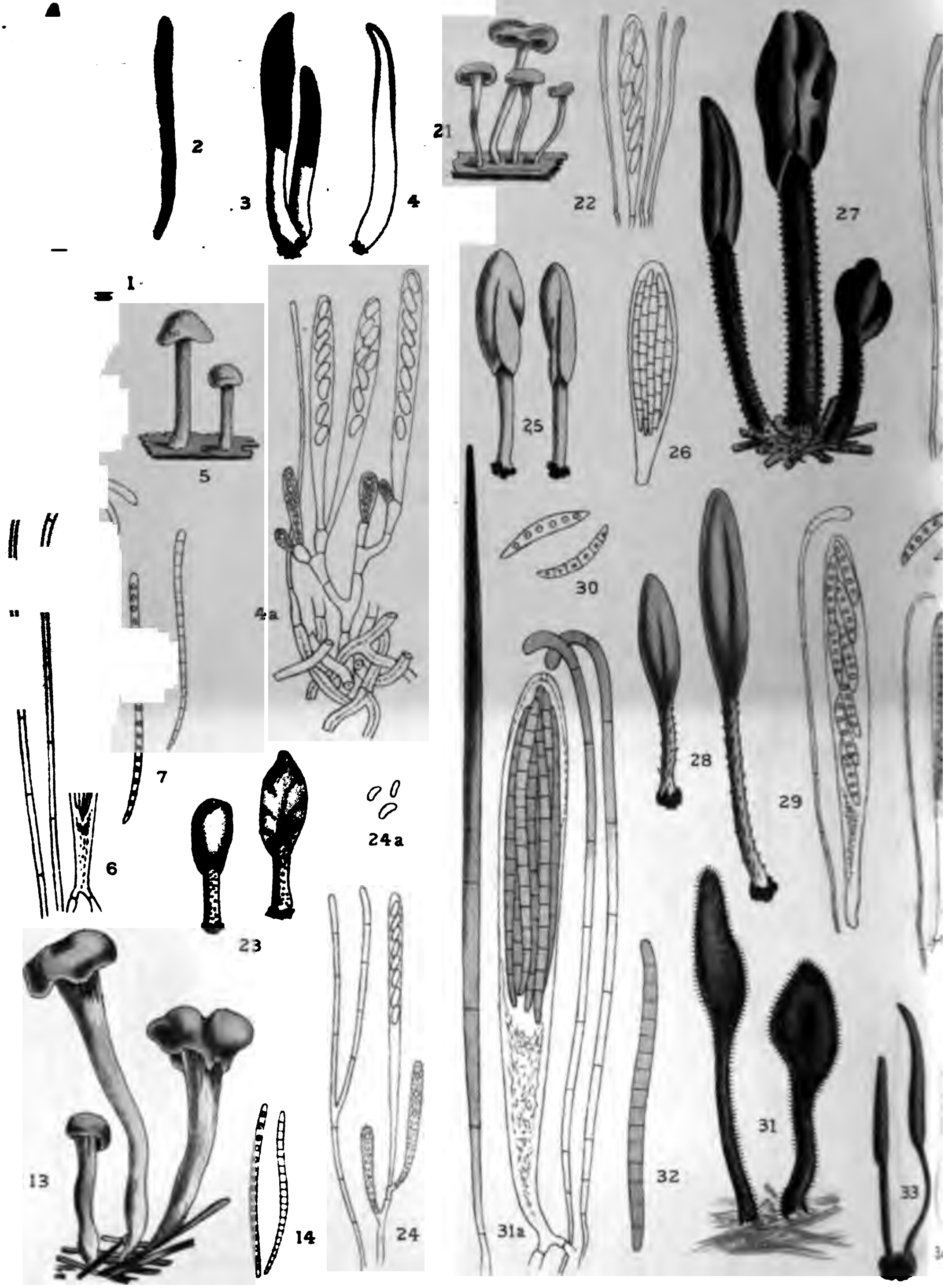

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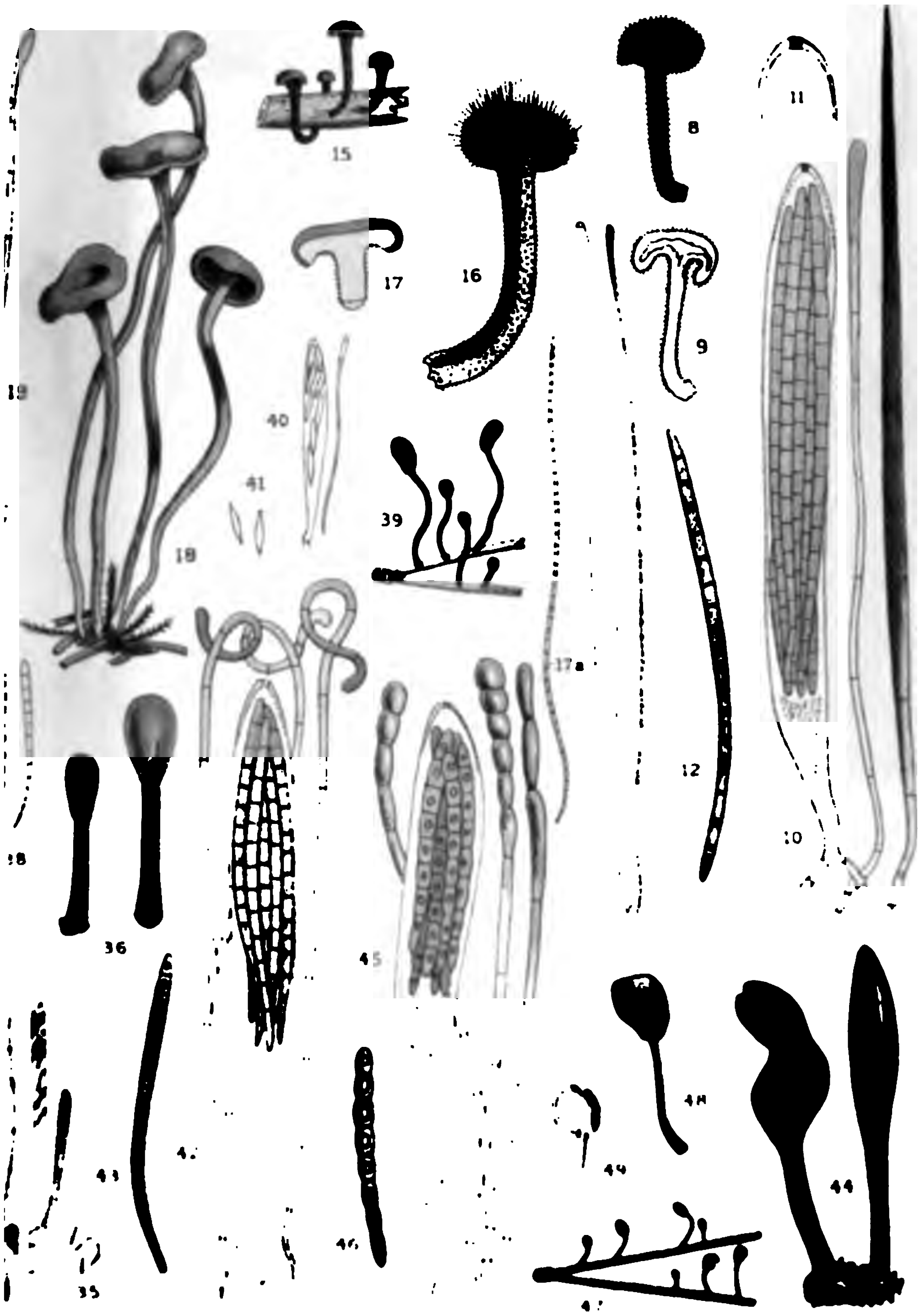
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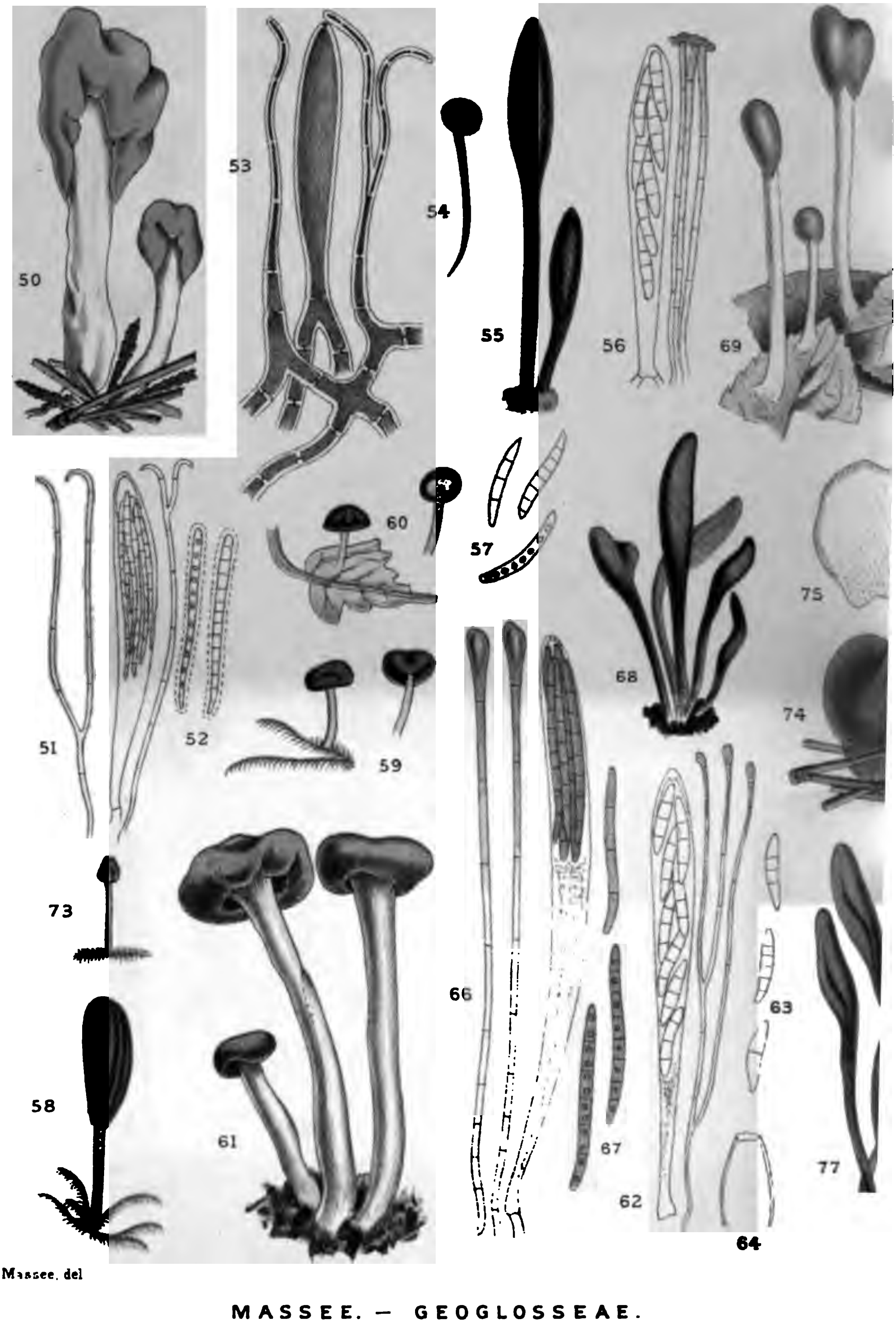

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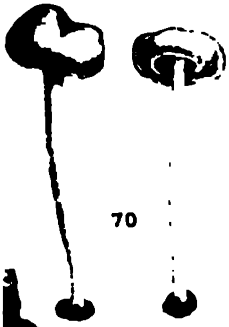
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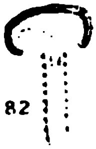
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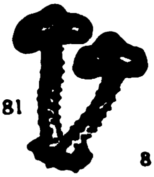
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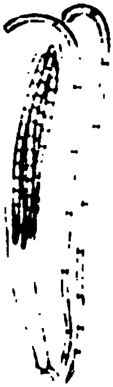
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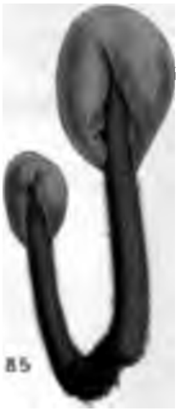
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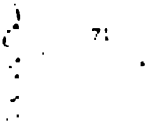
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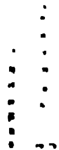
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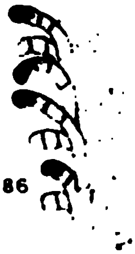
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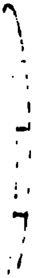
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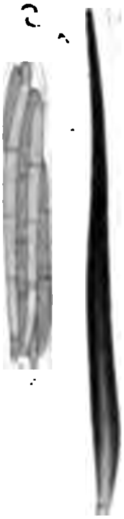

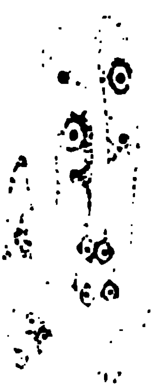

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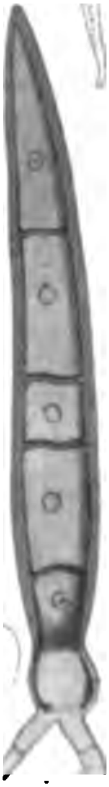
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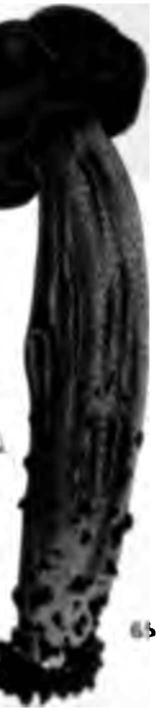
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On Polystely in the Genus Primula[1].

BY

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With Plate XIV.

SOME time ago the anomalous variations in structure which are found in the stems of the different species of the genus *Primula* attracted the attention of the French botanist, Van Tieghem, and they received an admirable elucidation at his hands. He showed that while some of the species are monostelic, others, on the other hand, exhibit a polystelic arrangement of their vascular tissue. However, being chiefly intent upon distinguishing between the various types of structure exhibited, and upon arranging the species according to these types, Van Tieghem did not pay much attention to the remarkable variations in structure which may occur within a single species, even in the different regions of one and the same plant, although these facts had already been observed and utilized for the purpose of classification

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[1] Van Tieghem, relying chiefly upon anatomical characters, has divided the genus *Primula*, L. into two separate genera, *Primula*, Tourn. and *Auricula*, Tourn. This separation agrees closely with that based upon the external characters which was established by Tournefort himself about 1700. However, it seems best to retain the customary nomenclature.

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in a paper written by Kamienski[1]. Believing that these variations may also be of some value for the purpose of indicating the manner in which the anomalous Primulas came by their remarkable structure, I purpose describing such cases as appeared in the species that I have examined.

To begin with, *P. japonica*, *obtusifolia*, *denticulata*, and *involucrata* are described by Van Tieghem as being gamostelic with a few steles only, and these arranged in a single circle[2]. The steles are two to four in number, elongate or arcuate in form, and they anastomose into a network, the meshes of which correspond to the points of insertion of the leaves. Although it is quite true that such a structure is frequently to be met with in these plants, yet at the same time it is by no means universally present; indeed, in the two first species, it can hardly even be described as generally characteristic. For instance, in the ten or more mature specimens of *P. japonica* that I examined, I did not find a single case in which the so-called steles maintained, throughout the plant, the essential features of true and perfect steles. On the other hand, both in *P. japonica* and in *P. obtusifolia*, the steles present in the stem are usually found to be defective or imperfect. These imperfect steles are at once to be distinguished from perfect ones by the more or less complete absence of those internal vascular bundles which are invariably to be found on the inner side of every perfect stele. In fact, they consist merely of a number of collateral vascular bundles with normal orientation, fused together laterally into so many groups each of which is surrounded by its own endodermis and pericycle (Fig. 1). The endodermis is clearly demonstrable right round the group, on the inner side as well as the outer. The pericycle on the inner side of the group is represented by 1–3 layers of parenchymatous cells directly intervening between the protoxylems of the vascular bundles and the internal endodermis; on the outer side it is greatly complicated by the development of a radiciferous network

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[1] Abhandl. der Naturf. Gesellschaft zu Halle, XIV, p. 143, 1878.

[2] Ann. Sc. Nat. Bot., 7 sér., T. III, pp. 300 and 305.

(*réseau radicifère*), which, both in the perfect and the imperfect steles, not infrequently takes the appearance of an outer series of normally oriented supernumerary bundles. This strange appearance is found wherever the elements of the radiciferous network are cut through transversely. The real nature of these outer bundles becomes at once evident in those sections which pass through the exact point of origin of a root; here it is seen that their position in the pericycle is taken up by horizontally running elements evidently connected with the root-stele, and belonging to the radiciferous network (Fig. 1; cf. also Van Tieghem, l.c., Fig. 31).

In four of the specimens of *P. japonica* examined, the imperfect type of stele alone was present throughout the whole length of the stem. In some it was present in the regions towards the base of the stem only, while the rest of the stem possessed perfect steles. In the remainder, although the imperfect form was present throughout the greater part of the stem, perfect steles also appeared in limited regions variously distributed.

Neither did any of the plants of *P. obtusifolia* that I examined show a perfect gamostelic structure throughout. In one plant the perfect steles did not appear at all; in the others, they were found only in limited regions situated in different parts of the stem.

The only point of difference between the imperfect steles found in *P. obtusifolia* and those in *P. japonica* is that in the former, owing to the strong sclerosis undergone by the cells of the central region of the ground tissue, the endodermis on the inner side could not always be identified with certainty, although it is distinctly recognizable in young specimens.

In *P. obtusifolia* and *P. involucrata* those parts of the stem which are formed at the end of one season's growth and the beginning of the next do not attain more than about one third of the full diameter of the stem, and in these regions the number of steles present is always reduced to three, two, or even a single one, shaped like a horseshoe. Further, they invariably seem to be imperfect, showing a complete absence of internal bundles. The internal endodermis is readily observed in *P. involucrata*, though not (for reasons mentioned above) in *P. obtusifolia*.

Imperfect steles of a slightly different kind are also to be found in *P. involucrata* and *P. denticulata*, but, although they are constantly to be met with in all regions of the stem, the result is not so far-reaching and disturbing in its effect upon the general anatomy of the plant as in the case just described for *P. japonica*. The chief point of difference between the imperfect steles found in these two plants and that in *P. japonica* is that here the internal vascular bundles are absent only from the median portions of the stele, while they are present for some little distance around each corner. In the median region, where the internal bundles are wanting, the internal endodermis drops in towards the xylem of the external bundles, until it is separated from their protoxylems by one or two layers of pericycle only (Fig. 4).

The existence of this kind of imperfect stele is explained by an examination into the manner in which the leaf-traces take up their place in the ring or circle of vascular tissue in the stem. They do so at the lower angle of each mesh in the network of steles, coming in between two perfect steles as they approach each other towards the lower end of the mesh (Fig. 2). There the leaf-trace fuses laterally, at the same time, with both of these steles (Fig. 3) so as to form a large arc, in which, of necessity, the internal vascular bundles which should be present just within the leaf-trace are wanting, while those on the inner side of the two perfect steles which are connected by the leaf-trace arc, of course, present (Fig. 4). However, as this imperfect stele passes down the stem, internal bundles appear in the median region also, on the inner side of those bundles which came in from the leaf-trace, and thus the large arc becomes a perfect stele. Lower down still it divides by constriction into two smaller, but at the same time perfect, steles, which are now ready to undergo a similar process again with a lower leaf-trace (cf. the steles in Fig. 2).

In these several cases, it is certain that the absence of the internal bundles and the consequent production of imperfect steles has nothing whatever to do with the production of the monostelic terminal floral peduncle: for plants have been examined which possess the imperfect type of stele throughout a given year's growth, itself terminating in a floral peduncle, and also exhibiting the same structure again in the next year's growth immediately above the peduncle.

Among the dialystelic species I examined *P. Auricula* and *P. Palinuri*, and as regards their mature structure they agree in every respect with Van Tieghem's description. The steles are perfect throughout, although a slight trace of the phenomena described in connexion with the entrance of the leaf-trace in *P. involucrata* may sometimes be discerned; but only in relation to the median one of the many traces which enter from the leaf.

STRUCTURE OF THE PETIOLE.

In all the species that I examined, the vascular strands which pass off from the stem into the leaves are rather large in size, and take the form of arcs of greater or less curvature. In these plants at any rate, I have obtained a striking confirmation of the opinion so strongly advanced by Strasburger, that the leaf-traces take with them, from the stele of the stem, not only portions of the endodermis and pericycle, but also of the pith and sometimes of the primary medullary rays[1]. This fact is particularly well shown in *P. involucrata* and *P. obtusifolia.* The term 'meristele,' therefore, as applied to these vascular strands, is especially appropriate, since they really include portions of each of the constituent parts of the steles from which they arise. The leaf-trace usually takes away several vascular bundles from the stem-stele, although they are not always to be separately distinguished in the meristele itself. The endodermis of the meristele, whenever it is clearly defined, is most certainly continuous throughout with that of the stem-stele from which the leaf-trace arose. In *P. Auricula* and *P. Palinuri* each leaf is supplied with several meristeles which arise from so many different steles in the stem. In other cases a single meristele arising from one of the stem-steles is given off to each leaf, and this meristele generally branches as it passes outwards so that several are to be found in the petiole. The structure exhibited by such petioles may therefore be fitly termed 'meristelic.' The median meristele is always the largest and the most curved, sometimes forming as much as two-thirds of a complete circle (*P. obtusifolia*). The lateral meristeles become smaller and less curved as they are the more distant from the median one.

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[1] Histol. Beit. Vol. iii, p. 110

Van Tieghem describes the petioles of all the species of the genus *Primula* as being astelic throughout their whole length[1]; that is to say, they contain either a single meristele or several separate and distinct ones. The only exceptions to this statement that I found were in the petioles of *P. japonica* and *P. denticulata*, where certain structures are invariably present, which in all essential respects are entirely similar to normal and perfect steles. These steles were found in all the specimens examined of both species, and they extended from a point near its base throughout the petiole, and often for a long distance along the midrib of the lamina also. Of the several vascular strands in the petiole, only the three median ones form steles, the lateral strands being meristeles similar to those described above.

These petiolar steles are circular in transverse section, and consist of a ring of phloëm surrounding a ring of xylem. A considerable amount of parenchyma is scattered among the elements of the xylem, but separate vascular bundles are not distinguishable either here or in the phloëm (Fig. 6; cf. Fig. 5). The centre of the stele is occupied by a sclerotic parenchyma, scattered among the peripheral cells of which are the elements of the protoxylem, generally rather crushed and flattened. The central parenchyma is, in the median stele, generally marked off as a distinct medulla, but in the smaller steles it is either scanty or absent. Both in *P. japonica* and in *P. denticulata* the whole stele is surrounded by an exceptionally well-marked endodermis enclosing a collenchymatous pericycle, the latter consisting of about three or four layers towards the lower surface of the leaf, and rather less towards the upper.

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[1] Ann. d. Sc. Nat. Bot., 7 sér., T. III, p. 294; and also Bull. Bot. Soc. de Fr., XXXIII, p. 95.

In order to account for the appearance of these steles in the petiole, I will now describe the changes that the leaf-trace undergoes during its course. In these two plants the leaf-trace invariably arises from one of the steles in the stem as a single large meristele. It is at first curved into the shape of a horseshoe, open towards the interior of the stem, and is completely surrounded by the endodermis, which is folded round the corners of the horseshoe, following the concavity of the inner side. Within the endodermis lies the pericycle, and the concavity between the latter and the xylem is occupied by a few conjunctive cells continuous with the pith of the stem-stele. As the meristele passes through the cortex of the stem it branches so as to form several strands, the extreme lateral branches remaining meristeles throughout the petiole. As the three median strands (to which alone I now refer) are followed in their outward course, the endodermis is seen to straighten out across the gap between the arms of the horseshoe, gradually falling less and less into its concavity (Fig. 5). At the same time, the vascular tissue at each corner gradually extends itself across the gap so as to meet that approaching from the other side, and thus to complete the circle, so that finally a complete stele is the result. Again, towards the tip of the lamina the steles gradually lose their internal bundles in an inverse but perfectly analogous manner, becoming meristeles once more.

Although I do not wish to lay too much stress upon the value or necessity of a strict continuity of tissues, yet it should be noted that these steles are essentially different from the single central stele described by Van Tieghem in the petioles of the Cucurbitaceae and Solanaceae. In the latter, as Strasburger points out, the tissue which Van Tieghem regards as pith is really only enclosed cortical tissue, and is not continuous with the pith of the stem at all.

In spite of the discovery of these polystelic petioles in the Primulas, the fact that the Gunneras are more deeply imbued with the phenomenon of polystely still holds good; for they possess polystelic floral peduncles also, while those of the Primulas are monostelic. As regards the possession of polystelic petioles, I was at first under the impression that these two genera stood alone among the Phanerogams. But, from an interesting paper recently published by M. Casimir de Candolle[1], I gather that in certain species of *Alchemilla* (*A. vulgaris*, &c.) the petioles contain structures precisely similar to those described above, and which I take to be steles also. This resemblance becomes the more remarkable on account of the fact that another feature, cited by M. de Candolle as characteristic of the Alchemillas, is also excellently demonstrated in certain Primulas (*P. involucrata*, *obtusifolia*, and *japonica*), namely, that in these plants the vascular system of the axillary shoot is not directly connected with one of the stem-steles, *but with the leaf-trace itself*, joining on to the latter some distance before it takes its place in the central ring of vascular tissue in the stem.

Considering these facts as a whole, it appears that in *P. japonica* and *P. denticulata* a leaf-trace is capable of transforming itself into a stele at any point in its course whenever it may be advantageous to do so. Thus, if such a leaf-trace be followed throughout its downward course, at first, in the upper part of the lamina, it is found to be a perfectly collateral meristele. Towards the base of the lamina, or at any rate throughout the petiole, it exercises the capability which it possesses of producing internal bundles on its upper side, and thereby converts itself into a perfect stele. On arriving at the periphery of the stem it again returns to its collateral structure, and enters into the vascular circle as a horseshoe-like meristele. Later, passing down the stem, the now cauline portion of the leaf-trace once more develops internal bundles, and in connexion with the cauline portions of other leaf-traces with which it has fused, it becomes once more a complete and perfect stele. Finally, it divides into two branches, which eventually fuse with the leaf-traces of the lower leaves as they take their places in the vascular circle.

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[1] Bull. de l'Herbier Boissier, T. I, No. 10.

Bearing in mind the manner in which the leaf-trace enters the vascular ring between two steles already present, as described for *P. denticulata* and *P. involucrata*, and also the general course of a leaf-trace as followed out above, it seems possible to regard the whole vascular system of these plants as consisting of the cauline portions of the leaf-traces. They pass in, arrange themselves in a circle, and having passed some distance down the stem, divide into two branches, which insert themselves on the entering leaf-traces of the lower leaves. The fact that the leaf-trace has become a stele during the cauline portion of its course may be regarded as incidental only. For such an incident may, and does, take place at other points of its course where no very great importance can be attached to its occurrence. Moreover, the examination of *P. japonica* and *P. obtusifolia* has shown that whether the trace becomes a stele or not, *even in the stem itself*, is just as much a matter of chance, or rather of convenience

Regarded in this light, the existence of the imperfect steles in *P. japonica*, *obtusifolia*, *involucrata*, and *denticulata* is easily understood. For the appearance of the internal bundles on the inside of the cauline portion of the leaf-trace may either be more or less delayed (*P. involucrata*, *P. denticulata*), or they may sometimes be altogether dispensed with (*P. japonica*, *P. obtusifolia*). Again, in the two latter plants their presence in certain varying regions of the stem, and their absence in others, is also to be expected, for the leaf-trace may be regarded as potentially capable of producing internal vascular tissue at any point throughout its course. Their actual existence in any particular region would thus depend upon the physiological conditions to which that region is exposed.

In *P. Auricula* and *P. Palinuri*, although the leaf-trace produces no internal vascular bundles at any point in its foliar course, yet they are invariably present in the cauline portion, appearing as soon as the leaf-trace turns downward in the stem.

THE LEAVES OF THE SEEDLING.

The leaves of the seedlings of these plants exhibit, as regards their structure, an interesting series of gradations through which the leaf-traces become gradually less and less complex as the cotyledons are approached. In those species in which the petiole of the adult form of leaf is polystelic (*P. japonica*, *P. denticulata*), the first step that takes place is the reduction of the steles to meristeles throughout the whole length of the petiole, which takes place at about the seventh leaf. Then the leaf-trace ceases to give off lateral branches, so that the petiole contains one meristele only. Finally, this meristele itself diminishes in size until it appears simply as a single small collateral bundle. In *P. involucrata* and *P. Auricula* similar changes take place: but since the traces in the adult form of leaf itself are only meristeles, the first step that takes place in descending to the earlier leaves in the former is a reduction in the number of branches given off by the median trace, and in the latter a reduction of the leaf-traces given off from the steles of the stem. In *P. Auricula* the traces are at first reduced to three, and then, at the first or second leaf above the cotyledons, the leaf-trace in both species is represented by a single small bundle only.

These gradations indicate progressively the various arrangements employed by the individual plant to provide the amount of vascular tissue necessary for the steadily increasing needs of the leaves. And, as far at least as the later steps are concerned, they probably represent phylogenetically the several devices by which the ancestors of the plants endeavoured to attain a similar end. We may therefore suppose that, as a first resort, the original vascular bundle was enlarged to the greatest size that such a collateral structure could possibly attain compatible, at the same time. with economy of space. This point reached, it became subdivided into several strands. Then, in *P. japonica* and *P. denticulata*, a still further increase in the amount of vascular tissue present was obtained by the production of bundles on the inner side of certain of the traces, thus converting them into perfect steles.

TRANSITIONAL PHENOMENA IN THE SEEDLING.

The epicotyledonary region of the stem in these plants is monostelic, with a narrow pithless central cylinder, in which no separate vascular bundles can be clearly distinguished. The structure remains the same also for some little distance up the stem, except that the central cylinder increases slightly in diameter owing to the appearance of a few pith-cells at its centre. Two different types of development are to be found in the seedlings of *P. japonica* which are parallel to the two types of structure present in the mature stem. Seedlings exhibiting the first type would no doubt grow into those mature plants in which the imperfect form of stele is at least greatly predominant, if not exclusively present. In these the central cylinder of the epicotyl is retained in a perfectly normal condition until the departure of the seventh or eighth leaf-trace at least; in fact, so far as the differentiation of the tissues renders any decision possible. The first three or four traces in passing out leave a small gap of two or three cells only, and although the higher traces may leave a somewhat wider gap, still the endodermis invariably passes directly across the opening, and does not become folded into the interior of the cylinder (Fig. 7). Thus the gap does not really affect the stele as a whole, but only the ring of vascular bundles. In fact, it is only an unusually large medullary ray which becomes filled up again by the more or less rapid growth of the vascular tissues on either side to meet each other across it. This type is by far the most prevalent; the other, which probably corresponds to those plants in the mature structure of which the perfect stele predominates, was present in two cases only out of fourteen examined.

In the second type of seedling the central cylinder is, as before, maintained intact until the third or fourth leaf, but the gap formed by the departure of the fourth or fifth trace becomes an actual gap or break in the cylinder itself. The endodermis, instead of passing straight across, falls in around the margins of the gap, and passes right round the periphery of the medulla, just within the xylems of the vascular ring (Fig. 8); so that when this gap becomes closed up by the approximation of its margins, the endodermal layers first meet and fuse (Fig. 9), then the pericycles, and finally the ring of vascular tissue again becomes continuous. But now that portion of the endodermis which has been invaginated into the medulla, in consequence of the process just described, has become cut off and separated from the external endodermis, and forms a distinct internal endodermis, which often also extends into the internode below the gap at which it was actually invaginated (cf. Fig. 10). When the next leaf-trace departs, the internal endodermis is once more put into continuity with the external endodermis around the corners of the gap. Further up the stem they become separate again in exactly the same way as before. The seedling has therefore attained at this point exactly the same structure as that termed (for the sake of convenience) imperfect gamostely in the mature plant.

According to Van Tieghem, almost identical processes are gone through in the seedling of *Botrychium Lunaria*[1], resulting in an exactly similar structure, which, however, he here speaks of as 'gamodesmy.' If it be held necessary to retain Van Tieghem's terminology throughout, I see no alternative to calling the imperfect steles 'gamodesmic groups,' both in the

[1] Journ. de Bot., Vol. iv, No. 23, p. 405.

seedling and also in the mature plant of *P. japonica* (cf. Van Tieghem, l. c., No. 24, p. 483).

From one point of view the chief peculiarities of this type of structure may at the same time be epitomized and explained by considering that the leaf-trace, when it enters into the circle of vascular tissues, carries the endodermis of its upper surface straight in with it through the breadth of the vascular ring, where this endodermis then turns down at the inner border of the trace and runs down the stem, spreading laterally at the same time so as to form the internal endodermis of the vascular ring or stele, as the case may be (Fig. 10).

In *P. involucrata* the epicotyledonary central cylinder is maintained until the seventh leaf, or even higher. The first two or three leaf-traces, including those of the cotyledons, produce a gap of about two or three cells wide, the higher ones of about six cells. In the latter the endodermis, in passing over the gap, becomes slightly concave; however, this infolding is not deep, and the endodermis soon straightens out again as the gap fills up (Fig. 11). When the seventh or eighth leaf-trace comes off, this infolding of the endodermis is carried on to a much greater extent, and at the same time the gap remains open for an unusually long distance; so long, indeed, that the next leaf-trace above is given off from the opposite side of the stem before it becomes closed (Fig. 12). Around the margins of this gap also the endodermis is very deeply infolded, so much so, in fact, that it meets and fuses with the endodermis infolded from the other side through the still unclosed gap of the seventh or eighth leaf-trace (Fig. 13). Later on they separate completely, thus dividing the vascular ring into two separate groups, each completely surrounded by its own endodermis and forming an incomplete stele or gamodesmic group. Proceeding upwards, these fuse across the earlier gap left by the seventh or eighth leaf-trace, to form a horseshoe-like group which remains open on the opposite side of the stem (eighth or ninth leaf gap) until the next leaf-trace above comes off (Fig. 14), which divides it in an exactly similar manner into two new groups, and so on.

Sometimes the transition takes place in *P. involucrata* much in the same way as in *P. japonica*, the endodermis at the seventh or eighth leaf-gap being completely invaginated, and cut off by the closing of the gap. As in *P. japonica*, the departure of the next leaf-trace establishes a horseshoe of vascular tissue. But here the gap between the arms of the horseshoe remains open until the departure of a still higher leaf-trace, which divides it into two groups, and then it proceeds as above described. In either case the vascular groups are at first imperfect steles, but after some time they develop internal vascular bundles, and assume the form found in the mature stem.

In *P. Auricula* the fact that even the earlier leaves are supplied with more than one leaf-trace from the stem serves to complicate matters considerably, but the median trace has such a paramount influence on the transitional phenomena that it alone is alluded to below.

The epicotyledonary stele is maintained intact until the fourth or fifth leaf (cotyledons included) is reached. Until this point a very small gap only is left by the outgoing traces, but at the departure of the fourth or fifth leaf-trace a gap of considerable width is formed, around the margins of which the endodermis is invaginated into the medulla, just as in *P. japonica*. Thus a horseshoe of vascular tissue is produced which remains open on one side until the next leaf-trace is given off on the opposite side of the stem, dividing it into two separate groups, just as in *P. involucrata*. These two groups are almost always of unequal size, and they appear to become perfect steles even before the next leaf-trace comes off from the largest of the two. So we see that *P. Auricula* becomes completely and typically polystelic at a very early stage. The lateral leaf-traces arise on either side of the median trace from the steles that have just been formed by the departure of the latter, or, in the case of the formation of the horseshoe, from its corners, whereby the gap between its two arms becomes much widened.

Van Tieghem seems to have entirely overlooked the all-important influence of the leaf-traces on the phenomena of the transition, and, indeed, on the vascular system throughout the whole plant. On this account he regards the polystely, when present, as having originated by the continued bifurcation of the central cylinder found in the lower part of the stem. He speaks of it as flattening itself out and constricting itself in the middle until it becomes nipped into two[1]. I have not seen anything that would lead to a similar conclusion. The transitional phenomena in the seedling, and also the extreme variability in certain species, of the most important characteristics of polystely, give strong support to the opinion that polystely is not a primitive feature of the group of Primulas in which it is found, but a comparatively recent modification. Monostely is undoubtedly to be regarded as the fundamental type, and Van Tieghem appears to be correct in laying so much stress upon the incapacity of the narrow epicotyledonary stele, in the polystelic section of the genus, to expand itself sufficiently to satisfy the needs of the plant, an inability which he regards as the ultimate cause of their polystely (l. c., p. 304).

Since the epicotyledonary stele of the monostelic section of the Primulas has retained its ability to expand with the growth of the plant, that of the polystelic section must have lost this same power, which at one time it also possessed, and it must have done so at a period anterior to the first appearance of polystely in the section. The only way in which this could have come about is by the prototypes of the polystelic section having been at one time subjected to very different conditions from those of the monostelic section, those conditions being such as were unfavourable to the production of much vascular tissue, and therefore favourable to the establishment of a very narrow central cylinder throughout the plant. Now a recurrence to those conditions, in which an increased amount of vascular tissue would be advantageous, could be met, as regards the leaf-traces, by an increase in their size or in their number. But if such an increase actually took place, it would appear that the epicotyledonary cylinder, having lost the faculty of adequate expansion, and being no longer able to enclose these leaf-traces within its slender circumference, would have to accommodate them in some other manner.

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[1] Ann. des Sc. Nat. Bot., 7 sér., T. III, pp. 282 and 295.

Now, we may reasonably regard the manner in which the epicotyledonary stele of the seedling becomes converted into the mature structure of the stem as a reminiscence of the phylogenetic progress of the race itself, though, of course, with the earlier incidents blurred by subsequent adaptation to the surroundings. It therefore appears probable that the narrow central cylinder, being unable to expand, became broken up, in connexion with its constituent leaf-traces, into so many vascular arcs, and that these arranged themselves in a ring of suitable diameter around the centre of the stem, fusing with each other laterally at intervals, and opening out again. In fact, a structure was produced similar to that found in some specimens of *P. japonica* and *P. obtusifolia* at the present time.

Again, it is evident that even such a ring as this might at length reach a limit of expansion, relative to the size of the stem, while room could still be found for more vascular tissue on the inner side of the cauline portions of the leaf-traces, thus converting them into steles, and at the same time without breaking up the ring. The various gamostelic species may be cited as exhibiting such a structure.

If, however, we suppose that a continual increase in the number or size of the leaf-traces entering the stem necessitated a still further increase in the amount of vascular tissue contained in the latter, there appears to have been no alternative but to break up the ring and to scatter the steles throughout the ground tissue, as is to be observed in many dialystelic species.

However that may be, one result appears clear—that, in the Primulas, the gamostelic condition is more primitive and nearer normal monostely than is the dialystelic type, and that probably a gamodesmic condition preceded either.

STRUCTURE OF THE APEX.

I found that in the apical region of these plants the vascular system undergoes no simplification whatever, but presents precisely the same arrangement as it does in the fully differentiated stem. Therefore, if in the fully differentiated regions there are several steles arranged in a slightly interrupted ring, as in *P. japonica* and *P. involucrata*, they will also be represented in the apex by a similar ring of desmogen-strands, which may be followed up as far as any differentiation can be distinguished in the meristem. Whether this apparent plerome extends past the youngest leaf, into the apical cone itself, is somewhat doubtful, but, on the whole, the probability is that it does so.

Again, if in the differentiated regions of the stem the steles are irregularly scattered and widely separated, as in *P. Auricula*, the desmogen-strands which correspond to them are also found to be scattered in the merismatic regions of the apex; and further, in this case it appears tolerably certain that no plerome is to be found in the apical cone itself. Therefore in these plants the study of the apical meristem throws no light upon the phylogenetic origin of the various types of structure; such light can only be obtained from the ontogeny of the whole plant as exhibited in the development of the seedling.

The most striking feature in the apical region is the extreme insignificance of the development of the apical cone itself compared to that of the youngest leaf produced by it. The latter, immediately after its first formation grows with great rapidity, completely overshadowing and pushing aside the minute apex which often becomes a mere lateral protuberance at the base of the precocious leaf. Correlated with this early and excessive development of the young leaves, the leaf traces also play a predominant part in the first appearance of the desmogen-strands in the meristem of the apical regions especially in *P. japonica* and *P. involucrata*, where by far the greater portion of the ring of desmogen is formed by the leaf-trace that has latest entered it, a fact completely in accordance with the manner in which the leaf-traces regulate the arrangement of the vascular system in the fully differentiated stem.

The greater part of this paper was prepared at the Royal Gardens, Kew, and I desire particularly to express my gratitude to Dr. Scott, the Honorary Keeper of the Jodrell Laboratory, for the interest he took in my work, and to thank him for the valuable advice I received from him at almost every point of the investigation. I have also to thank the Staff of the Gardens for the supply of material which they placed at my disposal.

EXPLANATION OF FIGURES IN PLATE XIV.

Illustrating Mr. Gwynne-Vaughan's paper on Polystely in the Genus *Primula*.

Note.—Figs. 1, 5, and 6 only are to be regarded as accurate drawings. The insertion of the leaf-traces and adventitious roots, and also the presence of the radiciferous network, cause so much confusion among the vascular elements that I was obliged, for the sake of clearness, to make the other figures somewhat diagrammatic, although in every case I follow the actual sections as closely as possible.

Abbreviations: *m.* medulla; *x.* xylem; *ph.* phloëm; *p.* pericycle; *e.* external endodermis; *i.* internal endodermis; *c.* cortical tissue; *l. t.* leaf-trace; *l. g.* leaf-gap; *s.* a stele of the stem.

Fig. 1. *Primula japonica*; portion of transverse section of an imperfect stele; *r.* the elements of the radiciferous network; *ext. b.* external bundles. The internal bundles are completely absent. × 130.

Figs. 2, 3, 4. *Primula involucrata*; portion of transverse section of the stem, showing a leaf-trace entering between two perfect stem-steles. × 40. In Fig. 2 the leaf-trace is seen approaching the steles; in Fig. 3 it has become fused with both of them. Fig. 4 shows the imperfect stele thus produced, which has no internal bundles in its median region.

Fig. 5. *Primula denticulata*; transverse section of the median leaf-trace in the petiole. The stele is not yet perfect, but the vascular ring is open towards the upper surface. × 90.

Fig. 6. *Primula japonica*; transverse section of the median leaf-trace in the petiole. The stele is here quite perfect, having passed through a stage similar to Fig. 5 lower down in the petiole. x 90.

Fig. 7. *Primula japonica*; transverse section of central cylinder of a seedling. The leaf-trace departs in a normal manner, leaving the stele intact. x 100.

Figs. 8 and 9. *Primula involucrata*; transverse sections of central cylinder of a seedling. The leaf-trace departs in such a manner as to leave an internal endodermis. (*P. japonica* also makes use of this method of transition.) Fig. 8, the endodermis is invaginated through the leaf-gap. x 70. Fig. 9, the leaf-gap becomes closed in such a manner as to include a portion of the endodermis which becomes internal. x 100.

Fig. 10. *Primula japonica*; diagrammatic longitudinal section through the central cylinder of a seedling. The endodermis passes into the interior of the stele through the leaf-gaps. The dark regions indicate xylem: the light, phloem; and the dotted line, endodermis.

Fig. 11. *Primula involucrata*; transverse section of the central cylinder of a seedling after the departure of one of the earlier leaf-traces. The stele is still intact. x 100.

Figs. 12–14. Transverse sections of the same, showing how the stele eventually becomes broken up. $l. t.^8$ the eighth leaf-trace; $l. t.^9$ the ninth leaf-trace; $l. g.^7$ the seventh leaf-gap; $l. g.^8$ the eighth leaf-gap. Fig. 12 shows the invagination of the endodermis through the seventh leaf-gap. x 100. Fig. 13 shows that the seventh leaf-gap is still open, while the eighth leaf-gap is almost free; its final departure will break up the stele into two portions. x 70. Fig. 14, the two portions are fused across the seventh leaf-gap; the eighth leaf-gap is still open, and the ninth leaf-trace is about to pass off. x 90.

Fig. 1.

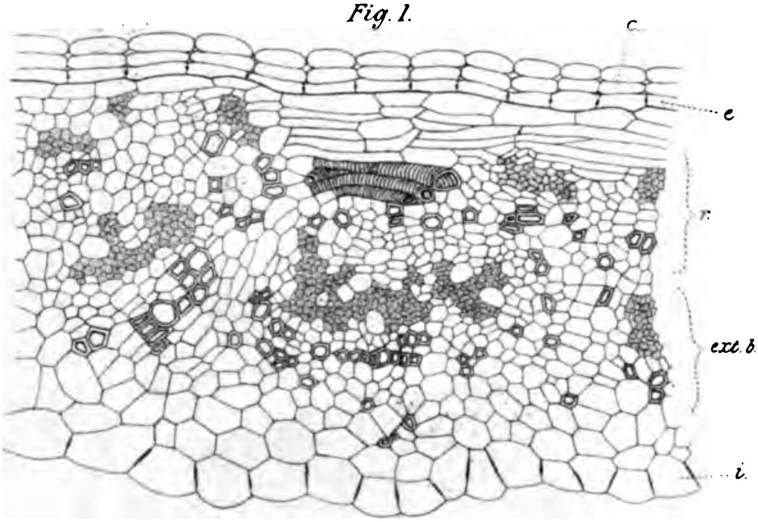


Fig. 2

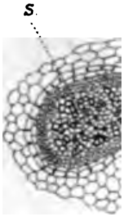


Fig. 6.

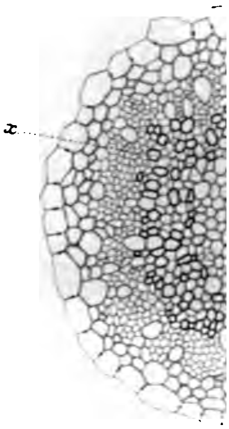


Fig. 5.

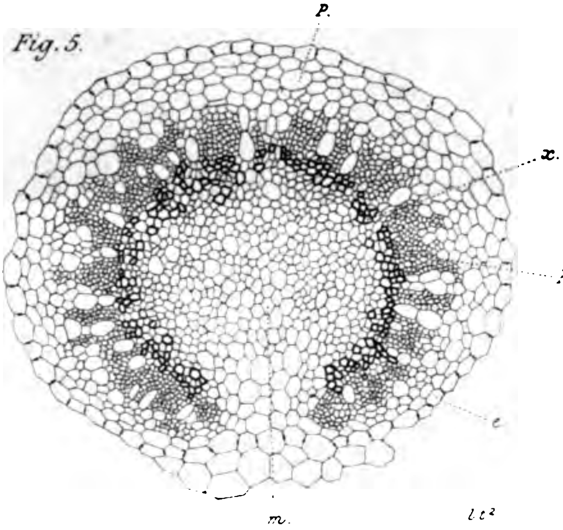


Fig. 11.

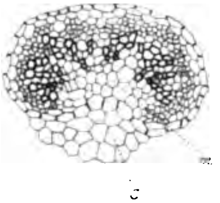


Fig. 12.

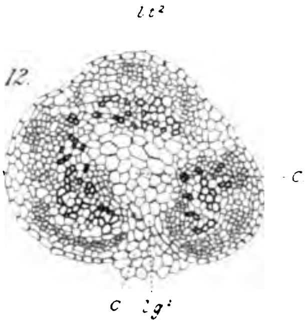
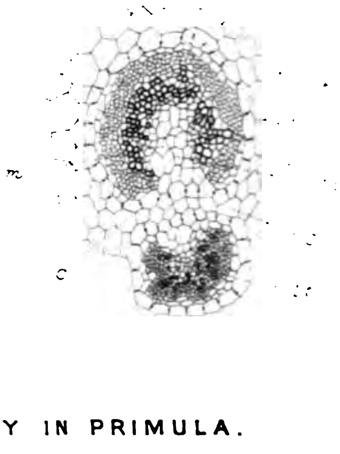


Fig. 7.



D. T. Gwynne-Vaughan. del.

GWYNNE-VAUGHAN. — POLYSTELY IN PRIMULA.

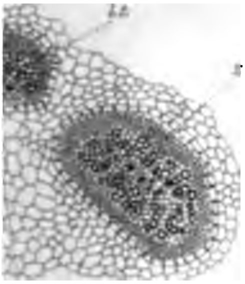


Fig 3

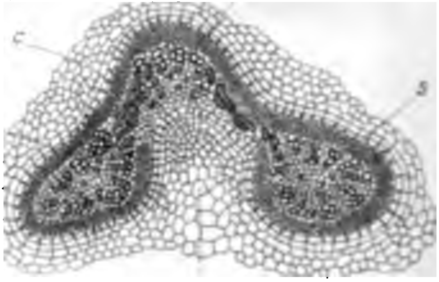
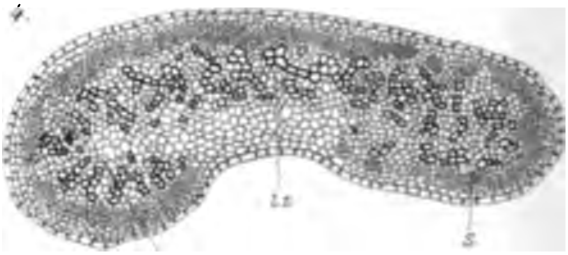


Fig 4



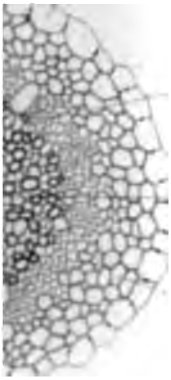


Fig 10

Fig 9

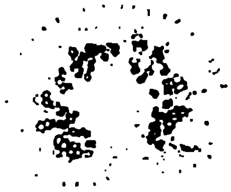
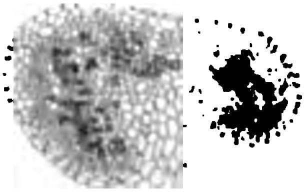


Fig 8

Fig [illegible]

Fig [illegible]



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On two new instances of Spinous Roots.

BY

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With Plates XV and XVI.

COMPARATIVELY few instances of spinous roots appear to have been recorded hitherto. Among the better known cases of the kind are certain Palms, such as the species of *Iriartea* and *Acanthorhiza*, in which the rootlets of the aerial roots are modified to form spines; and the Leguminous genus *Derris*, in which the adventitious roots themselves are said to become spinous, and to help in attaching the climbing stem to its support.

The object of the present brief communication is to place on record two remarkable, and as I believe novel, instances of spinous roots, which, curiously enough, have almost simultaneously come under observation at Kew, within the last few months.

Both the plants in question are Monocotyledons, but they belong to distinct Natural Orders, and are totally different in habit, the one being a *Dioscorea* and the other a *Moraea*.

1. DIOSCOREA PREHENSILIS, Benth.

The *Dioscorea* was raised from seed collected by Mr. Scott-Elliot in Sierra Leone, and presented to Kew in January, 1892. The plant flowered in September, 1894, and was then determined as the *Dioscorea prehensilis* of Bentham. The

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peculiarity of the roots was first noticed in February of the present year. The plant was at that time at rest, the leafy stems having withered. When dug up, it was found to have formed an irregular, lobed tuber about a foot long, which was enclosed in a sort of cage of hard, interlacing, spine-bearing roots, springing from the upper part of the tuber, the whole mass being about two feet in diameter (see Plate XV). At that time the normal nutritive roots, bearing ordinary rootlets, appeared to be produced only from the base of the stem, immediately above its attachment to the tuber.

It is important to notice that the whole system of spinous roots was produced entirely underground, so that their existence was not revealed until the soil was removed. That they form a most efficient means of protection to the parts which they enclose is evident from the photograph, and is still more vividly realized by any one who has attempted to handle the rigid and bristling mass. We may reasonably suppose that the thorny hedge of roots serves to guard the tuber—the great food-store of the plant—from the attacks of burrowing or digging animals.

The spinous roots attain a diameter of about a quarter of an inch. They have an irregular curved course, usually starting from the tuber in an upward direction, and then bending down. In the mature condition they are very hard and woody, and consist entirely of the vascular cylinder or stele, the whole cortex beyond the endodermis having withered, and only hanging on the roots in shreds. The spines, which are inserted at irregular intervals, averaging an inch or less, reach a length of about three-quarters of an inch. They are sometimes solitary, in other cases two or three are inserted together at the same level, and on the same side of the root. It occasionally happens that two adjacent spines are coherent, having a common base, which may much exceed the free points in length. The spines themselves are exceedingly hard, and usually very sharp.

The study of the anatomy shows plainly that the spine-bearing organs are actually roots, in which the vascular tissues are reduced as compared with the lignified prosenchyma. The spines themselves are shown by their structure to be rootlets, though highly modified. In them, as in the roots which bear them, the hardy woody portion is entirely stele, limited on the outside by the endodermis. The dry and withered cortex often forms a membranous envelope around the lower part of the spine.

Histological details are reserved for a future communication, which must await the opportunity for a study of the development. The spinous roots at present on the plant are not only mature, but to all appearance dead, serving no other than a purely defensive function.

The normal roots have the structure usual among Monocotyledons; it is of interest, however, to note that, when old, the rootlets die off, leaving behind a somewhat spiny base, so that there is here a certain approach to the peculiar character of the special protective organs.

The plant has now been transferred to a hot, moist house, where it has rapidly formed a new twining stem, already nearly thirty feet in length and half an inch thick. The stem, which for some time remained unbranched, is clothed with strong, broad-based prickles, which bear a marked superficial resemblance to the spines on the roots. Anatomical examination however, shows that the prickles contain no vascular elements so that they are no doubt merely outgrowths from the external tissues

At intervals averaging about fourteen inches, the stem bears thick, green cuspidate scale-leaves, two inches long, which are alternate near the base of the stem, but elsewhere are inserted in pairs. These curious organs, which are sessile, with a broad base, bear no resemblance to the normal foliage of the plant They probably represent the modified and enlarged leaf-bases. The stem is now (April 27) producing branches from the axils of the scale-leaves, but at present the branches, like the main stem, bear scale-leaves only, and have as yet formed no normal foliage[1].

[1] These have since appeared, i.e. branches of the second order. May 5

Numerous fleshy normal roots are growing vigorously from the base of the stem, and a few from the tuber itself. So far, however, there is no sign of any increase in the tuber, nor have any new spine-bearing roots as yet made their appearance.

The existence of spinous roots is not altogether unknown in the genus *Dioscorea; D. spinosa*, Roxb., is described in Hooker's Flora of British India (vol. vi, p. 291) as having 'long woody rigid fibres, bearing spines half an inch long.' Roxburgh himself, in describing the same species (under the name of *D. aculeata*), uses these words: 'tubers oblong, pendulous, the fibres of the proper roots become spinous[1].' It would appear, then, that this Indian species is similar, as regards the character in question, to the West African *D. prehensilis*. It is not improbable that other instances may be brought to light within the genus *Dioscorea*, which appears to offer considerable scope for further morphological investigation.

2. MORAEA, sp.

Root-clusters of this Iridaceous plant were sent to Kew, in December, 1896, by Mr. J. W. Mathews, of the Municipal Gardens, Cape Town, who found it growing wild in the neighbourhood of Cape Town. The stem has an enlarged base, perhaps representing the original corm; above the base it becomes cylindrical, and is hard and woody throughout. The spinous roots, which are stiff and wiry, spring from the swollen base of the stem on all sides, radiating out in every direction, and curving and interlacing, so as to form a dense network, which bristles with spines, and suggests a vegetable hedgehog more than anything else.

The whole mass is from two to three inches in diameter, and is not unlike the root-cluster of the *Dioscorea* on a small scale (see Plate XVI). The ordinary roots arise chiefly from the under surface of the enlarged base of the stem.

Histological investigation shows that the spinous roots are practically identical in structure with the normal absorptive roots. In both alike, the mature root consists chiefly of the vascular cylinder, on which the dead cortex hangs loosely; in both the endodermis is greatly thickened, and the conjunctive tissues of the stele much lignified. The only difference of importance is that the more internal vessels of the spinous roots are smaller than those of the normal organs.

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[1] Roxburgh, Flora Indica, iii, p. 800.

The spines themselves, which are often branched, are manifestly rootlets, having essentially the same structure as the roots which bear them, except that in the spines the vessels are still further reduced.

Numerous small corms, varying in size from that of mustard-seed to that of a hazel-nut, bud out from the basal part of the main stem, among the spinous roots, which perhaps serve more especially for their protection. The corms easily become detached, and afford an abundant means of propagation. Young plants are now being raised at Kew from some of these corms, so it may be hoped that the whole development of the plant, especially that of its remarkable root-system, may be followed as time goes on.

The fact that earth is present among the roots, in all parts of the cluster, no doubt indicates that the whole mass was developed in the soil. It would be interesting to ascertain whether in nature it ever becomes loosened from its attachment to the ground, and carried away by wind, like a 'Rose of Jericho.' It is possible that the corms, which separate so readily from the stem, may be distributed in this way.

I am indebted to the Staff of the Royal Gardens, Kew, and especially to Mr. W. Watson, Assistant Curator, for much information concerning these plants. The present preliminary communication is only intended to record the main facts as to the curious and exceptional forms of root illustrated in the photographic plates.

When opportunity arises I hope to enter on a full investigation of the development and histology of the organs in question.

EXPLANATION OF PLATES XV AND XVI.

Illustrating Dr. Scott's paper on Spinous Roots.

PLATE XV.

Dioscorea prehensilis, Benth. Tuber, with mass of spinous roots springing from and enclosing it. The normal roots are those arising from the base of the stem. About one-fifth of natural size.

PLATE XVI.

Moraea, sp. Base of stem, with clusters of spinous roots.
Fig. 1. Seen in section: on the left a corm is well shown.
Fig. 2. In surface view: almost natural size.

Both from photographs by Messrs. Gunn and Stuart.

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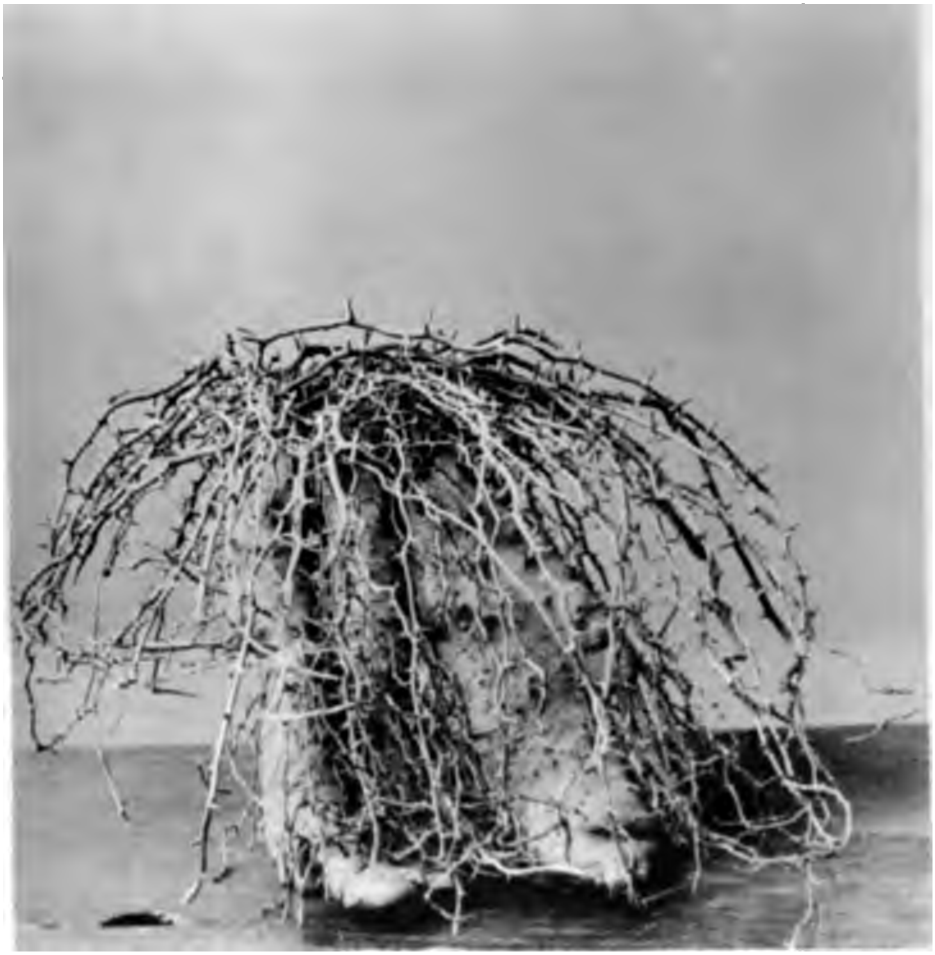


Dioscorea prehensilis, Benth.

SCOTT, ON SPINOUS ROOTS.

Fig. 1

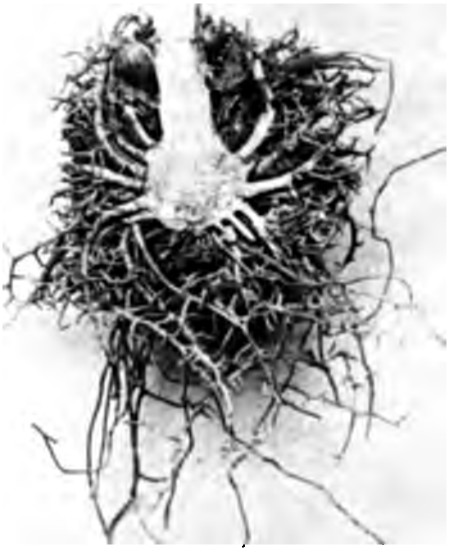
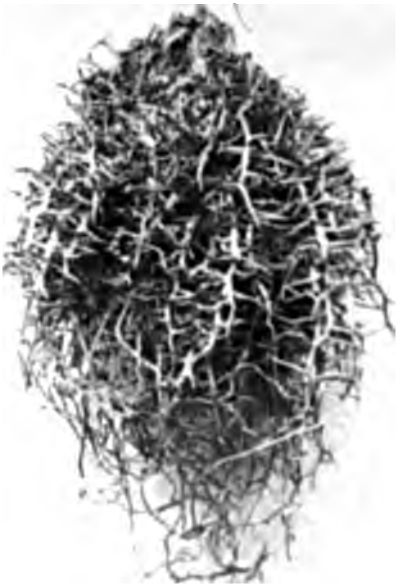


Fig. 2



SCOTT ON [illegible] ROOTS

NOTES.

FUNGI FOR CLASS-DEMONSTRATION.—The following may possibly be useful to others who, like myself, are concerned in the preparation of class-material.

Bunt.—To obtain material for *Tilletia*, I break up three or more bunted grains of Wheat in water in a small evaporating dish; weigh or count out the seed-wheat required and add it to the water (the amount of which depends on the quantity of seed used); stir thoroughly and carefully, and allow to dry, stirring frequently as drying proceeds, so as to get the spores well distributed instead of allowing them to settle where the film of water over the grain is in contact with the dish or with other grains. I usually do this in the afternoon, so that the grain is dry enough for sowing on the following morning.

The effect of a copper-sulphate dressing is easily shown by taking part of the Wheat thus contaminated and dressing it with copper-sulphate in the proportion used by farmers, and sowing it when dry in a row next my control row. In the past three years I have found no instance of the failure of the copper-sulphate dressing to prevent Bunt in Wheat. I keep my stock, for class-work on the spore-germination, in covered glass jars.

Smut.—The method adopted for Bunt was a failure as regards Smut. Nor did I obtain very great success by soaking and opening the seed-grain and actually placing the spores on the embryo. The plan I now follow is to soak Barley in water for about twenty-four hours: I then remove the chaff and stir up the naked kernels in a small amount of water, in which I have broken up several grains of smutted Barley; allow to dry, and sow when dry. My Barley tillers well, so to obtain material for the early stages of spore-formation, I pull up a plant on which one ear or more is smutted, and dissect out the ears of the eight or ten or more shoots that so far have no ears visible externally. Once I found a plant with two ears smutted and six or eight sound, but this was the only time that all the ears on a plant were not equally affected; and once I found an ear with the three lowest kernels alone smutted while all those above were normal. To keep up my stock of spores for germination in class-work, I gather the smutted ears before the spores are shed, and keep them in small tin tobacco-boxes. On one occasion a small beetle got shut up too, and was imprisoned for a week or two before I found and released it. I noticed many spores adhering in short threads, really the excreta of the said insect. I tried the germination of the excretal and normal spores in a culture-cell (made by fastening a glass ring to a slide and growing the spores in a hanging drop of water on the cover-slip inverted on the ring), and found the former to germinate in about half the time required for the latter. For this summer's material I smutted some Barley with spores taken from Barley (Row 5 of my plot at the Botanic Garden), some with spores from Oats (Row 7), and some with spores from Wheat (which, however, gave no result). Omitting details, I may say that I found 70–80 % only of the grain grew, and I gathered eighty smutted ears from Row 5 and forty-six from Row 7, amounting to 37 % and 21 % respectively of the total number of ears produced.

Phytophthora infestans.—As Potatoes are not to be absolutely depended upon, and as growing and infecting successive sets of potato-tops under bell-jars involves much space and time, I have for the past three years used *Solanum laciniatum* as class-material. I can rely on finding *Phytophthora infestans* upon it and in good condition, and its petioles and midribs furnish material much superior to potato-leaves. I prefer to gather all my *Phytophthora* material before the dew is off, and I keep it in a vasculum until my class at 11.0, when I use it fresh.

W. G. P. ELLIS.

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THE FUNCTIONS OF LATEX.—Although several attempts have been made to ascertain the functions of latex in plants, they have not led to any very conclusive information respecting it. Accordingly, at the suggestion of Prof. Marshall Ward, I undertook an examination of the subject from several points of view. My apology for bringing forward the results in so incomplete a state is that, at present, I have not the time at my disposal to complete it, owing to my departure for the tropics, and that it may possibly prove suggestive enough to excite interest in the problem and so help in its solution.

The work of Schullerus[1], Treub[2], and Faivre[3] is too well known to need description, as is also Schimper's[4] criticism of it.

Haberlandt's work[5] is disputed, but as he still maintains his original views in the last edition of his Physiologische Pflanzenanatomie, I have thought it worth while to repeat it.

In leaves in which the palisade-tissue is loosely packed, as in *Euphorbia mellifera* and *Euphorbia punicea*, frequent examples of the palisade-cells converging to the blind apex of a laticiferous cell, or a cell in direct connexion with it, can be met with.

In *Euphorbia spinosa*, where the palisade-layer is double, the branches of the laticiferous tubes run between the two layers and send out terminal branches into the upper layer of palisade-cells, thus forming a kind of brush-work. The blind endings are usually on the upper surface of the palisade-cells.

These terminations may be traced by mounting the sections in phenyl-hydrazine, the high refractive power of which renders it a very useful clearing agent. At the same time a marked staining action occurs in the palisade-cells and in the cells in which Fry[6] has described 'proteid aggregates,' which may be worth further investigation.

In laticiferous plants whose assimilation is carried on by the stem owing to partial or complete reduction of the leaf-surface, the assimilating cells form a compact layer several cells in thickness, e. g. *Euphorbia arborescens* and *coerulescens*. The laticiferous cells pass directly through this layer and end, for the most part, on the upper surface of the outermost layer of assimilating cells.

Taking all observed cases into consideration, I think we may safely say that the blind endings of the laticiferous system are generally connected with the palisade-cells.

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[1] Schullerus, Abhandl. d. bot. Vereins d. Prov. Brandenb., 1882.

[2] Treub, Anns. du Jardin Bot. de Buitenzerg, vol. iii, p. 37.

[3] Faivre, Ann. des Sci. Nat., sér. 5, t. x. p. 33. C. R. t. lxxxviii. p. 269, etc.

[4] Schimper, Bot. Zeit., 1885, p. 771 et seq.

[5] Haberlandt, Sitzb. d. k. Akad. Wien, 1883, p. 51.

[6] Fry, Annals of Botany, Vol. v, p. 413.

If it is improbable that a plant would build up so elaborate a system for the storage of waste products, it is possible that this method of terminating among cells in which assimilation is carried on may point to a storage or means of transit of reserve products.

This is further borne out by the extremely early appearance of the system in the leaf. A longitudinal section through a stem-apex of *Euphorbia pulcherrima*, for example, shows, in leaves just beginning to unfold, a well-developed system with its earliest branches extending to the palisade-layer.

Again, if latex is simply waste material, old leaves should contain large quantities of it, but this is by no means the case.

If young leaves and old leaves are lightly punctured, the young leaves exude far more latex than the old ones, and the same is true for young and old shoots.

Micro-chemical tests give the same results. If starch is present in the latex, a section of a young leaf shows the tubes well stored with it; while in an old or a fallen leaf it is difficult to find a trace, even if the iodine-method of staining is used. The starch has evidently been handed on from the laticiferous system to other parts of the plant.

Testing for proteids with Millons' reagent, or by the xanthoproteic reaction, gives completely parallel results. *Ficus Carica* is a possible exception, for here one occasionally finds granular masses in the tubes which give proteid reactions.

Though caoutchouc may be met with in fallen leaves, yet, from what is known of its chemistry, it is hardly likely that this substance has any nutrient value in the plant; but there is a possibility that, as do many other terpenes, it may absorb oxygen as ozone. When the endings of the tubes, and Schunck and Brebner's[1] demonstration of the presence of ozone in the palisade-layer, are taken into consideration, this view becomes more tenable. Direct proof of this has not been obtained, though testing latex with Wurster's papers and with mercury points to the presence of minute quantities of free ozone. I hope to obtain evidence upon this point, among others, while examining the india-rubber industry in East Africa.

To determine whether any physiological connexion exists between the laticiferous system and the assimilating tissues, chemical methods had to be applied.

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[1] Schunck and Brebner, Annals of Botany, Vol. vi, p. 167.

So far the chemical side of the question appears to have been badly neglected, although it was attacked so long ago by v. Mohl[1]. We may pass over the few analyses made of latex yielding economic products, and briefly notice that Weiss and Wiesner[2] have shown the presence of sugars in latex, and that Green[3] has examined its proteids.

As the sugars are so intimately connected with assimilation, it was decided to estimate them in latex drawn from plants under varying conditions with regard to their capability for assimilation.

The method employed was to take a known quantity of latex and remove the proteids by precipitation with excess of alcohol. After standing for a day, the solution was filtered off and evaporated to dryness over a water-bath. The sugars were then extracted from the resinous mass with warm water and estimated with Fehling's solution. The precipitate was weighed as CuO. With ordinary care this method gives very constant results, which is a point of considerable value when working with small quantities of latex. Where tannins are present they should be extracted with hide powder or ethyl acetate; but as the process leads to a certain amount of loss, it is as well to use latex free from them when possible.

We will first examine the results obtained when assimilation is checked by darkening the plants. Four plants of *Euphorbia pulcherrima* were placed in a dark room, and another set were kept in a greenhouse at a subtropical temperature.

The undarkened plants showed the presence of sugars equivalent to ·021 grms. CuO per c.c. After being darkened for a day the amount of sugar fell to ·002 grms. CuO per c.c., and after another day no trace could be found. *Euphorbia* [illegible] gave the same results.

Judging solely from the bulk of the proteid precipitate, the proteids had also undergone a considerable diminution in quantity.

If one may dismiss pathological changes due to abnormal conditions in this case, we may say that there is some definite connexion between the laticiferous system and the assimilating tissues. Further experiments confirm this conclusion; for if the plants are grown under normal conditions and the latex examined before and after the day's assimilation, an increase is found at the end of the period, as is shown by the following experiments made with *Euphorbia Peplus* in the early part of November, 1896, e.g.:—

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[1] Mohl, Bot. Zeit., 1843, p. 553 et seq.

[2] Weiss and Wiesner, Bot. Zeit., 1861, p. 41, and 1862, p. 125.

[3] Green, Proc. Roy. Soc., 1886, No. 242, p. 28.

Nov. 3, 1896, a bright sunny day—

Latex collected at 10 A.M. gave ·0302 grms. CuO per c.c.
" " " 4 P.M. " ·036 " " "

A sharp frost put an end to this series of experiments. The result of exposure to this cold was that the plants gave no exudation of latex on wounding even when brought into a warm room; on applying pressure a thin opalescent liquid was squeezed out.

Further experiments were accordingly carried out with *Euphorbia pulcherrima*, with the following results:—

Collected at 10 A.M.	*Collected at* 4 P.M.		
·032	·049	grs. CuO	per c.c.
·030	·046	"	"
·033	·046	"	"
·030	·047	"	"

Here an increase of sugars after the day's assimilation is evident.

With *Euphorbia arborescens* very discordant results were obtained:—

Collected at 10 A.M.	*Collected at* 4 P.M.		
·032	·032	grs. CuO	per c.c.
·034	·0346	"	"
·0185	·013	"	"
·012	·018	"	"
·021	·0195	"	"

The discrepancies in the foregoing results were found to be due to the fact that the latex had been drawn from different parts of the plant, the younger portions being richer in sugar than the older.

When we consider that some of the chief constituents of latex are starch, proteids, and sugar, all substances of undoubted nutrient value, and that the proteids are typical circulatory forms, and that the quantities of sugar vary with the plant's assimilation, it is evident that one function of latex is to carry reserve-materials in the plant.

Whether caoutchouc, resins, and tannins have any value, nutrient or otherwise, in the plant still remains to be determined. If they prove to be simply waste products, we shall be confronted with a peculiar method of dealing with waste matter which does not show much analogy to methods known at present.

R. H. BIFFEN.

FRANK SMART STUDENT,
GONVILLE AND CAIUS COLLEGE, CAMBRIDGE.

ON PEZIZA AURANTIA.—In March, 1896, the large pond in the Botanic Gardens, Cambridge, was emptied and cleaned, and large quantities of mud, chiefly the peculiar blue clay—gault—so common here, were removed from the bottom, together with plant-remains, such as the rhizomes and stems of water-lilies, *Epilobium*, *Scirpus*, *Polygonum*, &c. This mud was put into heaps in the garden under the shade of trees. At the same time a heap of similar blue clay, obtained during the digging for sewerage operations in the streets outside the gardens, was made near these heaps of pond-mud.

In September we noticed the apparently sudden outburst of enormous numbers of brilliant orange-scarlet *Peziza* cups on the heaps from the pond, and these increased during October and November, forming one of the most magnificent crops of these Fungi I have ever seen.

Examination showed the *Peziza* to be *P. aurantia* (Oed.), the cups, asci, and spores exhibiting the characters given in Phillips' British Discomycetes, p. 56, as those of the typical species, and reference to Massee (Brit. Fungus-Flora, iv, p. 448), Lindau (Engler, Pflanzenfam., 1 Th. 1 Abth. Lfg. 130, p. 187), and Winter (Rabenh., Die Pilze, B. III, p. 970) confirmed this, though these authors differ in the authorities cited for the name and synonyms.

Brefeld has not succeeded in germinating the spores of this species, and so I made attempts to cultivate it, and hoped to get out its life-history, but in vain.

It was quite easy to obtain the spores, though the majority of them appeared hardly ripe; nevertheless, numerous attempts to germinate them failed. In hanging-drops of water, gelatine culture-media, agar, &c., they did not stir under any of the given conditions, nor could I get them to grow in test-tubes on what experience suggested were probably suitable media.

At the same time, and especially when I found my attempts failing, I examined the Pezizas themselves to see if anything of the nature of a sclerotium or similar resting body had given rise to the crop; the results were negative. I also examined the plants round the pond for signs of such sclerotia, as well as the remains in the heaps, for it seemed possible that the sudden outburst of the crop on the particular heaps of mud from the pond might be due to large numbers of resting sclerotia having been dug up from the pond, although no such bodies are known in this group of Pezizas. All was in vain: no trace of any such body could be found either on the débris in the heaps or on the plants at the margins of the pond.

The observation that a few smaller patches of the *Peziza* occurred later in November on the clay heap not brought from the pond, made one ask whether, after all, it might not be merely a case of suitable pabulum, and to suggest that the blue gault clay was the favouring factor for the germination of wind-borne (or otherwise carried) spores.

Here again all experiments gave negative results, and I could not get the spores to germinate in either clay-washings or on the clay itself.

Moreover, I found that although the Fungus seemed to be spreading on to the sewer-clay, which was separated only by a narrow space —a couple of feet or so—from the heap of mud and débris of *Epilobium*, *Scirpus*, *Polygonum*, &c., where the large crop of Fungus grew, there was very little or none to be found on adjacent heaps of blue clay mud, also from the pond. On the other hand, a heap of the pond-mud and débris which had been fired to kill the *Polygonum*, &c., and covered with the same from the heap last mentioned, bore an abundant crop.

All these facts suggested that the hypothesis of wind-borne spores and a suitable pabulum was less probable than the hypothesis that the spores or other resting form of the Fungus had been brought from the pond with the mud.

Several times during repeated examinations of the heaps and their crop of *Peziza* cups, and of the latter when brought into the laboratory, I had noticed that the hymenium was marked with pale sunken patches, whence the asci and paraphyses had evidently been removed by some gnawing animal, and it required little search to find the culprit, in the shape of a small slug ensconced during the day on the lower side of the cup or on the ground near. The proof that the slug *was* the culprit was readily obtained by shutting it up in a glass dish in which was a piece of moist filter-paper with a *Pezisa* cup, for it rapidly attacked the hymenium in the dark, and gnawed just such holes in it as I had noticed.

In a few hours also the animal had left little ellipsoidal pellets of dung on the glass, paper, &c., and the bright scarlet colour of these told plainly the nature of his food. Microscopic examination of the dung-pellets showed abundance of spores, apparently uninjured, and now it seemed as if the problem was at length to be solved: of course it had suggested itself that the spores required passage through the body of the slug as a condition for germination.

Here again, however, nothing but failure attended all my efforts. It was very easy to obtain the spores, in the dung, in hanging-drops; yet in no case would they germinate, but behaved as if dead, until Bacteria obscured the view and the culture had to be abandoned.

Failure to germinate the spores has also attended every attempt made since the winter, in the hope that exposure to frost and a winter-rest might be necessary for germination, as is known to be the case with other spores.

H. MARSHALL WARD.

ON THE GINGER-BEER PLANT.—In my work on the Ginger-Beer Plant (Phil. Trans., B. 1892, p. 187) I pointed out the resemblances between Kephir and this symbiotic compound organism. On p. 186 I also gave reasons for believing that the *Bacterium* was introduced with the sugar.

I have now good reasons for believing that the early accounts of Kephir are not correct, or that there are several distinct varieties of this and other Ginger-beer plants, and that in all cases the Schizomycete I named *Bacterium vermiforme* is concerned, but associated with different yeasts; in any case, it appears certain that it can be artificially made to form a symbiotic union with other yeasts than the one I used in 1892; the aerobic yeast protecting the anaerobic *Bacterium*.

The following note is of interest in this connexion.

My wife recently received from a lady in Paris a number of grains of a body looking like boiled sago, and obviously of some such nature as Kephir or the Ginger-beer plant. It was said to have been given to our hostess by a missionary from Madagascar, who described it as 'an excrescence on the sugar-cane.'

It is used as follows. A table-spoonful of the Kephir-like grains is put into a litre-bottle—a champagne-bottle does well—with three table-spoonfuls of moist sugar (*cassonade*), and the bottle quite filled with water, securely corked and tied.

It is then left for three days, when a violent fermentation is found to be in play, so much CO_2 escaping that the pressure may burst the flask or blow the cork out violently if care be not taken.

The liquor is now strained into a second strong flask and securely corked down, the sago-like grains being returned with more sugar and water to the first flask to repeat the process. In four days it is ready for drinking, and is a slightly turbid, violently effervescing, lemonade-like drink, to which all kinds of curative and medicinal properties were ascribed by the missionary.

A microscopic examination of the grains show that they consist almost entirely of the sheathed form of a *Bacterium* so like *B. vermiforme* that I have little doubt it will prove to be the same. In much smaller quantity I found a yeast with long sausage-shaped pyriform and oval cells not very like *S. pyriformis*, and probably different from it.

On following the directions, I found the fermentation to occur exactly as described.

On carrying the experiments further, I was impressed by the particular stress laid on the direction to fill up the bottles and cork thoroughly, since this seemed to imply the necessity for keeping the organism out of contact with air. I therefore decided to try the following method. A bottle of Schweppe's soda-water was carefully opened, and a supply of ordinary sugar together with some of the 'Paris Kephir' (as I term it in my notes) added, and the flask, quite full, rapidly corked and tied. To my surprise the fermentation at once began, and all the sugar disappeared in a few days, the gas-pressure being tremendous.

The fermentation was almost entirely due to the *Bacterium*, very little yeast being present and apparently not increasing, and the conditions show that no oxygen is necessary to start the action.

Here we have clearly a case of an aerobic *Bacterium*, capable of fermenting sugar to carbonic acid and some other organic acid —the liquid has a pleasant acid flavour at the end of the fermentation —and requiring merely such traces of nitrogen as would be present in moist sugar.

Whether the soda-water and sugar will prove to be the best medium to cultivate it in or not remains to be seen, but it is clear from the five cultures I have made so far that the organism ferments very well under these circumstances.

I also desire to call attention to the following quotation from Cross and Bevan's book on Cellulose, 1895, p. 71, in this connexion, as it seems not impossible that the clots of 'white insoluble substance' there referred to may be this remarkable organism. On referring to Durin's paper in the Comptes Rendus—I can only find the second of the two papers mentioned—I find no evidence of any microscopic analysis of the clots, and it seems by no means unlikely that the existence of the micro-organism was overlooked.

'As a result of a change which is observed to be set up "spontaneously" in beet-juice, a white insoluble substance is formed, and separated in lumps or clots; this substance has all the characteristics of cellulose. After separating this insoluble cellulose, the solution gives with alcohol a gelatinous precipitate resembling the hydrates of cellulose previously described. These results are independent of the so-called viscous or mucous fermentations. That the process by which the cellulose is formed has the essential features of a fermentation-process, is seen from the fact that when the lumps or clots are transferred to a solution of pure cane-sugar or beet-molasses, a further formation of the cellulose ensues. When the process proceeds in neutral solution no carbonic anhydride is evolved; but in presence of acids this gas is evolved, and at the same time acetic acid is formed in the solutions.'

'E. Durin, by whom these phenomena have been investigated (Compt. Rend. 82, 1078; 83, 128), regards the ferment as allied to diastase, and states that fresh solutions of diastase itself act on solutions of sugar to form the soluble cellulose, precipitable by alcohol. There is also some evidence that cellulose may be formed from cane sugar in the plant by processes of this kind.'

The above, and several other problems connected with this interesting group of organisms, are now being investigated by Dr. Green in this laboratory.

But the proof that *B. vermiforme* is introduced with the sugar is all but complete. On referring to a paper by Koch and Hosaeus in the Cent. f. Bakt. B. xvi, 1894, p. 225, I find these authors discovered and figure a form evidently identical with mine, or so close to it that they were quite unwarranted in giving it a new specific name until they had cultivated it, especially as they knew of my work and pointed out the resemblances between their form and mine. They could not cultivate it by ordinary bacteriological methods, and were puzzled by the specimens being contaminated with a yeast. They do not seem to have tried anaerobic cultures, or to have really looked closely into my work; otherwise I cannot help thinking they would at least have tested the resemblances, amounting to identity as far as can be seen, to *B. vermiforme*.

My own impression is that their form was a case of wild Ginger-beer plant.

H. MARSHALL WARD.

SPERMATOZOIDS IN GYMNOSPERMS.—At the request of Dr. D. H. Scott, one of the editors of this Journal, we publish here in the English language a short *résumé* concerning the spermatozoids of *Ginkgo biloba* and *Cycas revoluta*, which we have lately discovered.

In *Ginkgo*, as well as in *Cycas*, the behaviour of the pollen-tube towards the archegonium is quite different from what we observe in all the Conifers investigated by Professor Strasburger and others. For the growing end of the tube, instead of elongating towards the neck-cells of the female organ, points towards the opposite direction, and produces in the nucellus, which is now a paper-like thin skin, many slender branches, which, acting like a root, serve to maintain the tube in that place. The other end of the tube, which is easily recognized as such by the remains of the exine covering it, produces within it, shortly before fertilization, two generative cells, each with a spermatic nucleus. Then an especially interesting phenomenon takes place, for here each of these cells begins to be metamorphosed into a spermatozoid.

The motion of the spermatozoid after its having broken out of the pollen-tube has been observed in *Ginkgo*; as to *Cycas*, however, its motion has not yet actually been observed; but the form as well as the development are so alike in both that now there is no reason to deny its motility. (As one of us has found that the fertilization in *Cycas* takes place at the end of September or the beginning of October, he intends in this year to prove its actual motility.)

The spermatozoid of *Ginkgo* is considerably larger than that of any spermatozoids of the lower and higher Cryptogams known till now, for it measures 82 μ in length and 49 μ in breadth. That of *Cycas* is larger than that of *Ginkgo* both in breadth and length. It is oval in shape. The head consists of three spiral windings in *Ginkgo*, and of four in *Cycas*, on which cilia, the organs of motion, are abundantly present. The tail is also formed, but is visible only after the element has broken out of the pollen-tube. Anatomically considered, it consists of a nucleus and cytoplasm, which covers it completely.

If we examine the ovules both of *Ginkgo* and *Cycas* towards the time of fertilization, we find a quantity of sap present between the apical concavity of the endosperm-body and the nucellar skin. This sap is necessary for the act of fertilization, for spermatozoids, by swimming there, are able to reach the archegonia. For it is a remarkable fact that in our plants the pollen-tube is far apart from the neck-cells of the female organ. As is well known, in other Gymnosperms the pollen-tube penetrates in fertilization more or less deeply into the archegonia, but in our plants the tube does not come at all into contact with the neck-cells. We think that this fact explains the reason why spermatozoids occur in *Ginkgo* and *Cycas* but not in other Gymnosperms, so long as the motile elements shall not have been discovered in such Gymnosperms, where the relation of the tube to the archegonia is different from that of our plants.

Since Hofmeister's classical researches, we know that there is no sharp boundary between the higher Cryptogams and the Phanerogams. On the other hand, however, it was generally accepted as a fact without exception, that in the former fertilization takes place by spermatozoids, while in the latter it occurs by pollen-tubes, so that we must have concluded that in this respect only there exists a sharp boundary between these two great groups of plants. But now this boundary has been broken down, and therefore the truth of the fact that both are very intimately connected becomes more and more prominent.

Finally we have to draw the attention of our readers to the fact that Hofmeister long ago stated his supposition that in the pollen tube of Conifers spermatozoids would be found to be produced. Pringsheim also repeatedly stated his opinion that they must be present in the pollen tube of Phanerogams. The supposition of these great botanists has now been proved to be partly right.

S. IKENO AND S. HIRASE,
Tokyo.

On the Development of the Cystocarp in Rhodymeniales.

BY

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With Plates XVII and XVIII.

IN the classification of the Florideae proposed by the late Professor Schmitz ('89), the Rhodymeninae constitute a group of six families, viz.:—the Sphaerococcaceae, the Rhodymeniaceae, the Delesseriaceae, the Bonnemaisoniaceae, the Rhodomelaceae, and the Ceramiaceae. This arrangement has been adhered to in Engler and Prantl's *Pflanzenfamilien* ('96), the parts of which on the Rhodophyceae are now appearing under the names of Schmitz, Falkenberg, and Hauptfleisch. In this work the Rhodymeniales, as they are there called, are thus distinguished:—Auxiliary cells for the most part differentiated only after fertilization of the carpogonium: the mother-cells of the auxiliary cells are disposed with the carpogonium in pairs, usually constituting procarps: after conjugation with the egg-cell, the auxiliary cell grows out into gonimoblast-filaments.

In former numbers of the Annals of Botany I have already published ('95 and '96) the results of observations on a series of genera of Rhodomelaceae, and I have since been engaged in extending these observations to the other families of the

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cohort Rhodymeniales, more particularly the closely allied Ceramiaceae. I desire now to record the results of observations on the structure of the cystocarp in the following species:—

Bonnemaisoniaceae: *Bonnemaisonia asparagoides*, C. Ag.
Rhodymeniaceae: *Plocamium coccineum*, Lyngb.
Sphaerococcaceae: *Calleblepharis ciliata*, Kütz.
Ceramiaceae: *Antithamnion Plumula*, Thur.
Griffithsia corallina, C. Ag.
Griffithsia setacea, C. Ag.
Callithamnion byssoides, Arn.
Callithamnion granulatum, C. Ag.
Ceramium tenuissimum, J. Ag.
Ptilota plumosa, C. Ag.
Plumaria elegans, Bonnem.

BONNEMAISONIA ASPARAGOIDES, C. Ag.

The family Bonnemaisoniaceae is represented in British waters by a single species, *Bonnemaisonia asparagoides*, C. Ag. By J. G. Agardh the genus was made the type of the Bonnemaisonieae, one of the four tribes of the order Chondrieae. The Chondrieae of Agardh ('63) no longer exists in the system founded by Schmitz on the structure and development of the cystocarp. The single genus constituting each of three Agardhian tribes have been removed to widely separate families: *Polyides* has gone to the Rhizophyllidaceae, *Solieria* to the Rhodophyllidaceae, *Lomentaria* to the Rhodymeniaceae. The tribe Bonnemaisonieae may be said to have survived in Schmitz's Bonnemaisoniaceae, shorn however of the large genus *Laurencia*, which has gone to the Rhodomelaceae. I cannot find that the minute structure of the cystocarp in *Bonnemaisonia asparagoides* has previously been described and figured. The description of the family in the *Pflanzenfamilien*, as far as it applies to this genus, certainly leaves much to be desired. The authors (Schmitz and Hauptfleisch, '96) regard the Bonnemaisoniaceae as intermediate between the Sphaerococcaceae and Rhodomelaceae, and nearest to the tribe Calleblepharideae of the former family.

It was therefore with considerable interest that I passed from an investigation of Rhodomelaceae to *Bonnemaisonia asparagoides*, the material for which I obtained from the Marine Biological Laboratory at Plymouth, where it had been collected and suitably preserved by Mr. George Brebner.

Superficially, the plant presents in its external ceramidia, a strong likeness to the more slightly corticated forms of Rhodomelaceae. Even its marked monoecism finds its counterpart in *Polysiphonia byssoides*, Grev. The ceramidia of Rhodomelaceae are however oblique, while those of *Bonnemaisonia* seem to be placed symmetrically on the end of the axis. The absence of tetrasporangia further distinguishes it from most Rhodomelaceae; and, combined as it is with monoecism, recalls the condition in many Nemalionales.

The investigation of the intimate structure of the young procarps is attended with considerable difficulty on account of their minuteness. After several trials, the most satisfactory method was found to be prolonged treatment with strong glycerine saturated with Hoffmann's blue, and the subsequent examination of the procarps whole.

I now propose to trace the history of the development of the cystocarp, as far as I have been able to decipher it. I omit all reference to the external morphology of the plant, which is well figured by Harvey, and to the anatomy of the vegetative parts which can be readily made out in material swollen in glycerine. Suffice it to say that the fertile branches occur on each side, alternately with the sterile branches, and so that a fertile branch is regularly opposite a sterile branch, a condition which occurs also in *Ptilota* and *Plumaria* among Ceramieae. When the branch which is destined to bear a procarp is seven or eight cells long and while peripheral cells are being cut off from the older cells in the plane of development of the plant, it is found that from the fifth cell onwards it is deflected towards the axis by the more rapid growth of the external peripheral cells of that joint. The proximal cell of the row of peripheral cells thus arising on the fifth joint, now begins to give off cells in all planes, which are bound together in a common mucilaginous mass and constitute a small cup. The deflected axial row shares, on the internal (ventral) side, in the formation of this cup. The second cell of the external peripheral row now gives rise above to a two-celled carpogonial branch, which occupies the hollow of the cup and extends beyond in an unusually short trichogyne (Pl. XVII, Fig. 1). A considerable proportion of the procarps which reach this stage do not develop further, presumably through failure of fertilization. In those which do, two things soon become marked. First, the rapid growth of the filaments constituting the cup, until this body becomes urn-shaped with an apical pore. The lower cells cut off, externally, cells which form a cortex to the pericarp, continuous with the cortex of the stalk. Secondly, the interior of the urn becomes filled with a compact hemispherical mass of tissue, the cells of which radiate from the base upwards and are of a bright-red colour (Pl. XVII, Fig. 2). At first, I considered this tissue to be the gonimoblast-filaments originating in an auxiliary cell. I discovered however that they gradually disappeared again in successively older cystocarps, and that the real gonimoblast-filaments arose much later, and consisted of larger and stronger filaments. These true gonimoblast-filaments surround the first crop of sterile filaments (Pl. XVII, Fig. 3), which become gradually more and more attenuated, until in maturer cystocarps they could not be traced. Reverting to the origin of the sterile filaments, I found, to the best of my belief, that they arise from the first cell of the carpogonial branch, the cell, that is, which lies directly beneath the carpogonium. By their luxuriant growth, the carpogonium itself is thrown over upon the base of the cavity of the cystocarp, where it may be seen at this stage, distinguishable only by its mucilaginous wall running out into the remains of the trichogyne. The origin of the gonimoblast-filaments is in a single cell arising from the base of the cavity, and derived from the same central cell from which emanate the filaments constituting the pericarp. This cell is the auxiliary cell, and there is no difficulty in understanding how conjugation may take place between the carpogonium lying against the base of the cavity, as I have described, and this auxiliary cell arising later in its immediate neighbourhood.

Comparing now the cystocarp of *Bonnemaisonia* with those of Rhodomelaceae, several points of dissimilarity are apparent. First, while axial cells share in the formation of the cystocarp in both, these occur on the inner (ventral) side of the organ in *Bonnemaisonia*, and on the outer (dorsal) side in Rhodomelaceae. Secondly, the carpogonial branch is 2-celled in *Bonnemaisonia*, if the hypogynous cell which proliferates is included: in Rhodomelaceae it is invariably 4-celled. Thirdly, while sterile filaments are found in the cystocarp both in *Bonnemaisonia* and Rhodomelaceae, in some cases, e.g. *Dasya*, among the latter even filling the young cystocarp as in *Bonnemaisonia*, yet in *Bonnemaisonia* these sterile filaments are formed exclusively from the hypogynous cell, and in Rhodomelaceae from two branches of the pericentral cell which gives rise to the carpogonial branch. Fourthly, no such paranematal filaments line the cavity in *Bonnemaisonia*, as invariably occur in Rhodomelaceae.

On the other hand, the carpogonial branch is at first external as in Rhodomelaceae, and the apical pore is formed in much the same way as in that family. In both these respects, *Bonnemaisonia* contrasts strongly with Sphaerococcaceae and Rhodymeniaceae, where the procarps are imbedded from the first, and the apical pore arises by dissolution.

I look forward to obtaining opportunities of examining representatives of extra-British genera of Bonnemaisoniaceae: at present, however, I cannot but think, judging from *Bonnemaisonia* alone, that the isolation of the family by Schmitz is fully justified, and that the relationship to the other families of Rhodymeniales is not so close as has been represented

PLOCAMIUM COCCINEUM, Lyngb.

This plant occurs commonly all round the British coast, and female plants with cystocarps in all stages of development may readily be collected during the early months of the year. Schmitz ('83) has given two figures of the early stages of the procarps, which are, however, insufficient for the complete understanding of the structure of the cystocarp. It was in order to obtain an insight into the history of the development of the fructification in a species of Rhodymeniaceae, that I undertook the examination of this readily accessible species. Schmitz and Hauptfleisch ('97) represent it however as a somewhat aberrant genus, presenting among Rhodymeniaceae the only case with a distinct axial row of cells. Its sympodial growth, and its stichidia with zonate tetraspores, undoubtedly mark it as a special form among Rhodymeniaceae, and may account for a cystocarpic structure somewhat different from that described by Hauptfleisch ('92) for *Chylocladia* and *Lomentaria*, and by Hauptfleisch ('92) and Davis ('96 *b*) for *Champia*.

Plocamium coccineum has all its parts flattened in one plane, a circumstance which makes it easily mounted by collectors. It is on the edges of the young branches that the procarps occur in great numbers. The method, which I have now for some time resorted to, for the examination of the more solid Florideae, is to cut either the fresh or appropriately fixed material in frozen gum arabic, by means of a microtome. This method has, I find, been elaborated by Osterhout ('96), and employed with success by several investigators.

The 3-celled carpogonial branches arise in the cells intermediate between the large medullary cells surrounding the axis, and the small cells constituting the superficial layer (Pl. XVII, Figs. 4, 5). I was not able to discover that the cells giving rise to these branches stood in any definite relation by genesis to the cells of the axial row. The branches incline obliquely forwards, and occur in great numbers, more particularly on the ventral edges of the thallus. As they take up stain readily, they may be easily detected in sections prepared as I have described. The three cells making up the branch are curved sharply in a crescent on the outside of the cell from which they are derived; so much so that two cells and the proximal portion of the carpogonial cell are contiguous to it. The trichogyne is usually somewhat inflated before it emerges at the surface.

The first step which follows upon fertilization is the growth outwards, from the mother-cell of the carpogonial branch, of a cell which is in still closer juxtaposition with the carpogonium. This is doubtless the auxiliary cell, as from it later all the sporiferous filaments are derived. In the figures of Schmitz, to which allusion has already been made, it is the mother-cell itself which is described as the auxiliary cell. In the posthumous work ('97) prepared by Hauptfleisch, the Rhodymeniaceae are described, in contrast to the Gigartinaceae, as perfecting the auxiliary cell subsequently to fertilization. In this case it arises only after fertilization. That this later-formed cell is the auxiliary cell is probable for this reason. From all analogy, a cell playing so important a part in reproduction is not likely after conjugation to give rise to sterile filaments as well as to gonimoblast-filaments. Now this mother-cell during the formation of carpospores gives rise to an abundant tissue constituting the inner layers of the hemispherical cystocarp. Davis indeed ('96*b*) in his description of the cystocarp of *Champia* regards several cells as auxiliaries, some of which are figured as sharing in the formation of the pericarp. He, however, shows that these cells do not give rise to gonimoblast-filaments, and indeed, he only designates them auxiliary cells on the supposition that they are the cells which Hauptfleisch has called by that name. In the sense in which Schmitz and Hauptfleisch use the term, the only auxiliary cell in *Champia* is the one which gives rise to the glomerulus of spores. But to return to *Plocamium*. When the auxiliary cell has been cut off, the carpogonial branch may still be seen, the carpogonium being even larger than at the moment of fertilization of the trichogyne

(Pl. XVII, Fig. 6). The trichogyne has by the time disappeared. The wall of the auxiliary cell is already highly mucilaginous, and the filaments lining the cystocarpic cavity are beginning to be pushed back. A later stage (Pl. XVII, Fig. 7) shows the auxiliary cell grown out into a tier of cells, branching on all sides at each tier. The terminal tufts of gonimoblast-filaments (gonimolobes) seem to mature first; those near the base, at any rate, seem less vigorously developed than those near the apex. The pericarp has now bulged out into a globular mass, and the cystocarp has become more or less external on the thallus. At the same time, the looser lining layer has assumed the appearance of a mass of attenuated filaments radiating from the base. Later an apical pore arises by dissolution of the wall, and the maturer carpospores escape.

In the circumstance that the auxiliary cell is derived from the mother-cell of the carpogonial branch, there is a clear resemblance to the Rhodomelaceae. There are, however, no sterile threads comparable with those of Rhodomelaceae and *Bonnemaisonia*. Other Rhodymeniaceae are, however, described as forming the auxiliary cell from cells contiguous to the mother-cell of the carpogonial branch, and even as forming more than one auxiliary cell (Hauptfleisch, '92).

CALLEBLEPHARIS CILIATA, Kütz.

As this plant occurs commonly on our shores, it seemed to me a readily accessible species of Sphaerococcaceae in which to follow the development of the cystocarp.

The procarps occur in great numbers along the margins of the 'cilia,' from which the plant derives its name. When one of these matures into a cystocarp, the 'cilium' becomes swollen, sometimes symmetrically, more often obliquely, so as to throw the apex over to one side. The cystocarp does not emerge as a globular mass upon the surface, but remains at best a broad-based protuberance.

The 3-celled carpogonial branches arise from a layer corresponding to that from which they arise in *Plocamium*. They seem to be distributed equally over the marginal surface of the pinnae; and the trichogynes, when they reach the surface, which many apparently do not, do so at varying inclinations, though they are mostly directed forwards (Pl. XVII, Fig. 8).

No auxiliary cell can be distinguished at the moment of fertilization any more than in *Plocamium*, and a considerable interval elapses before any gonimoblast-filaments can be detected.

The first effect of fertilization is to induce a rapid growth in a group of cells lying over the carpogonial branch, and derived from the mother-cell of this branch. They become greatly enlarged, stain readily, and give rise to radial rows of cells which cause the papillar elevation which is the first outward sign of fertilization (Pl. XVII, Figs. 9, 10). These might easily be mistaken for cells directly concerned with spore-formation. At this stage, the carpogonial branch may still be seen lying below apparently unaltered. A second effect of fertilization is the formation of a complex of small-celled filaments below and around the carpogonial branch. Before this has however attained any considerable development, there arises a tuft of gonimoblast-filaments between these inner and outer tissues marked in glycerine-material by the swollen cell-wall so characteristic of these sporogenous filaments. The basal cell of this tuft I regard as the auxiliary cell, and in position it is closely contiguous to the still traceable carpogonium. I could not decide, however, whether the cell which gives rise to the auxiliary cell is the mother-cell of the carpogonium, or the cell next above it. If, as I am inclined to believe, the auxiliary cell arises from the mother-cell itself, there is a close correspondence with the case of *Plocamium*. Schmitz and Hauptfleisch speak of the auxiliary cell merely as a 'closely contiguous cortical cell' As I have said, it is probably more than this, viz. a sister-cell to the carpogonial branch. Such however is the complex of placental 'rhizoids' in the neighbourhood of the tuft at this stage, that I failed to decide this point, though I examined many sections.

In the later history of the cystocarp the large-celled outer tissue loosens and gives way before the enlarging gonimoblast-filaments, forming the cystocarpic cavity. The small-celled tissue becomes a kind of placenta lining the base and sides of this cavity.

There would seem to be close relationship between Sphaerococcaceae and Rhodymeniaceae, and a remoter connexion between both families and Rhodomelaceae.

ANTITHAMNION PLUMULA, Thur.

It is to the Ceramiaceae, standing as they do, by general consent, nearest to the Rhodomelaceae, that I have devoted most attention; and among the species of Ceramiaceae which I have examined, none exhibit a simpler structure in the cystocarp than *Antithamnion Plumula.*

The carpogonial branch arises on the proximal joints of the pinnae close behind the apex (Pl. XVIII, Fig. 11). The branch arises laterally, its cells are smaller than the ordinary vegetative cells, and it curves round the joint-cell upon which it is produced, so that the carpogonium itself rests upon it above, and the long trichogyne stretches forward so as to overtop the densely aggregated branches near the apex of growth. The carpogonial branch is not free, but is throughout adherent to the joint-cell from which it is derived.

Fertilization having taken place, the fertile joint-cell, or basal cell, as it may be called, cuts off a lateral cell above in close contiguity with the carpogonium. Of the conjugation which ensues between these cells, I have no doubt, as I have seen the stout ooblastema-tube more than once, and demonstrated its presence to others (Pl. XVIII, Fig. 12). In a species of *Callithamnion* in which Schmitz ('92) observed this conjugation, he describes it as being effected by means of a small cell cut off from the carpogonium. Though I have not been able to satisfy myself of the existence of any cell-wall in the carpogonium, yet invariably a small mass of granular matter remains over in the carpogonium lying near the origin of the trichogyne.

This conjugation effected, the auxiliary cell forthwith grows luxuriantly into gonimoblast-filaments, made up of several gonimolobes. As this growth proceeds, the cells immediately above and below the auxiliary cell become fused with it. The pits widen out until the limits of the separate cells become indistinguishable. This fusion does not however extend beyond the basal cell below.

There is clearly great similarity between the process here described and that occurring in the Rhodomelaceae. In both cases, the mother-cell of the carpogonial branch is the proximal cell of a peripheral row, the carpogonial branch is 4-celled, the auxiliary cell is cut off from the basal cell above, and the basal cell gives rise to other sterile derivatives. In short, if the favella of *Antithamnion*, together with the basal cell and its sterile derivatives, were all included in a common pericarp derived from the axis, the condition of things in most Rhodomelaceae would be reached.

It would seem therefore that the Rhodomelaceae are a highly specialized family, whose ancestry is to be sought among the simpler Ceramiaceae.

GRIFFITHSIA CORALLINA, C. Ag., and GRIFFITHSIA SETACEA, C. Ag.

The procarps of *Griffithsia corallina* have been described by Naegeli ('47), and more fully by Janczewski ('76). The fertile branches consist of three axial cells: the lowest of these gives rise to the whorled involucre; the second also gives rise to a circlet of three branches, one 1-celled, and two 2-celled; whilst the third remains without further development. The proximal cell of each of the 2-celled branches arising from the second axial cell generates laterally a 4-celled carpogonial branch, of much the same appearance as in *Antithamnion* (Pl. XVIII, Fig. 13). Fertilization having taken place, an auxiliary cell is cut off the basal cell above, with which the carpogonium is in close contact. I have seen again in this species evidence of the existence of an ooblastema-tube connecting these cells, and I have no doubt that conjugation takes place (Pl. XVIII, Figs. 14, 15). The auxiliary cell then buds out into the favella. Miss Smith ('96) has recently described the cystocarp in *Griffithsia Bornetiana*, Farl., and shows that the single procarp which is there produced corresponds, cell by cell, with one of the procarps of *Griffithsia corallina*; and further, that no cell within the involucre, other than the auxiliary, shares, as had been previously supposed, in the production of the spores. She finds however no evidence of a conjugation between the auxiliary cell and the carpogonium, and hints that fertilization may take place by way of the cells of the carpogonial branch and the basal cell, regarding the fusion which takes place among the cells of the carpogonial branch as favouring this view. I have observed a similar fusion in *Griffithsia corallina*, where however I am satisfied that direct conjugation takes place. In this plant too, there subsequently takes place a very general fusion, including the second axial cell and the whole of its products, even extending upwards to the third axial cell. When this occurs, the mass however retains for some time the outline which belonged to it before fusion, and the genesis of the carpospores may still be seen to be confined to the situation of the two auxiliary cells. This fusion is no doubt to be associated with the increased demands for nutrition consequent upon rapid spore-formation. It is in accordance with modern ideas to suppose that all these spores derive their nuclei from the nucleus of the auxiliary cell, and it is observable that though fusion takes place, the spores are not formed equally from all parts of the fused mass. As I have said, it may be clearly seen in *Griffithsia corallina* that spore-formation is confined to the situation of the auxiliary cells. The auxiliary cells do not lose their identity, the other cells are probably utilized for their nutrition. Analogous processes occur in the ovule of Angiosperms, when the embryo-sac grows at the expense of neighbouring cells of the nucellus; and in the embryo-sac itself, when the embryo grows at the expense of cells of the endosperm.

In *Griffithsia setacea*, the fertile branches differ in structure from those of *Griffithsia corallina* and *Griffithsia Bornetiana*. They consist of an axial row of five cells. The first two contribute to the formation of the involucre, which here consists of two whorls. The third has no lateral outgrowths. The fourth gives rise to a whorl of branches of which one generates from its proximal cell a typical carpogonial branch. The fifth has no further development. Though I did not pursue the development of the favella in this species, I believe it arises in the same way as in the other species.

It would thus seem that, while the procarp itself and the development of the favella correspond closely in these species of *Griffithsia* with that already described for *Antithamnion Plumula*, yet each presents such marked peculiarities of structure in the fruiting-branch taken as a whole, as to make it possible, one would suppose, to establish readily distinguishable sub-genera, if nothing more, on this basis in a revision of the genus. Considering the great similarity, amounting almost to identity, sometimes existing in the structure of fertile branches between different genera of Ceramiaceae (e.g. *Ptilota* and *Plumaria*), it is remarkable that Naegeli's proposal to found the genus *Heterosphondylium* to receive *Griffithsia corallina* and *Griffithsia Schousboei* has not been adopted by later writers.

CALLITHAMNION GRANULATUM, C. Ag., and CALLITHAMNION BYSSOIDES, Arn.

The procarps of different species of *Callithamnion* and allied genera have been described and figured by Bornet and Thuret ('76 and '80), Janczewski ('76), and Schmitz ('83). The cells of which they consist are however so small and closely packed that it is difficult to make out their genetic relationship, so much so that Schmitz ('92) amended his earlier description on the ground that all other previous descriptions, including his own, had been inadequate. This amended description was not however accompanied by a figure.

From among several species of *Callithamnion* proper which I have examined, I give here the early condition of the procarp in *Callithamnion granulatum*, and one of a later condition in *Callithamnion byssoides*.

The carpogonial branch in *Callithamnion granulatum* appears to consist of four cells which are included in a common gelatinous envelope with the joint-cell from which it is derived. It is probable however that the branch is 3-celled, and that the fourth cell is a sterile derivative of the proximal cell of the branch. The joint-cell also buds out two cells right and left of the carpogonial branch (Pl. XVIII, Fig. 20).

In *Callithamnion byssoides*, the older joint-cells are much elongated, and the favella is lax, approaching the condition described by Bornet as seirosporic (Pl. XVIII, Fig. 21). The figure represents a stage immediately subsequent to fertilization, all the procarpial cells being still recognizable.

The interest consists in the fact that two cells have been derived from the carpogonium, right and left, and are in apposition with the second cells of the branches from which the two-lobed favella is derived. Schmitz, in the later description to which I have referred, speaks of the cutting off from the carpogonium of a small cell, by means of which the conjugation is effected. The figure shows a pair of such cells. Again, it is clearly the second cells of the sporiferous branches that are the auxiliary cells. First, because the ooblastema-cells are in contact with them only, and secondly, because no sporiferous threads occur below this point on the branches.

Judging from the case of *Callithamnion byssoides*, the genus seems to represent a specialized condition among Ceramiaceae, differing from the simpler condition represented by *Antithamnion Plumula* and other species in the 3-celled carpogonial branch; in the formation of two auxiliary cells to each carpogonium; in the occurrence of these auxiliary cells as the second cells of lateral branches; and in the conjugation between the carpogonium and these auxiliary cells being effected by distinct ooblastema-cells.

CERAMIUM TENUISSIMUM, J. G. Ag.

As is well known from Janczewski's description ('76), the genus *Ceramium* presents a case which contrasts in an interesting way with that presented by *Callithamnion*. While in *Callithamnion* the procarps consist of one carpogonial branch with two auxiliary cells, in *Ceramium* it consists of one auxiliary cell and two carpogonial branches. Both are equally specialized divergences from the more primitive type, but in quite different directions.

The plant whose procarp I have figured (Pl. XVIII, Fig. 19) is, I believe, *Ceramium tenuissimum* J. G. Ag., a very delicate diaphanous species. I neglected, however, to collect tetrasporiferous plants, by which the plant is more readily distinguished from its congeners. From the figure it will be seen that the auxiliary cell is not, as Bornet and Janczewski have supposed, the mother-cell of the two carpogonial branches, but a later-formed superior cell. The branches are 4-celled, as is very generally the case in Ceramiaceae. The trichogyne has been shut off in the procarp on the right in the figure, and the remains of the ooblastema-tube by which the carpogonium and the auxiliary cell had been put in connexion was seen as represented. No conjugation seems to have taken place in the case of the second carpogonium.

The case of *Ceramium*, apart from the duplication of the carpogonial branches, is again very similar to the primitive condition described for *Callithamnion Plumula*.

PTILOTA PLUMOSA, C. Ag. and PLUMARIA ELEGANS, Bonnem.

Plumaria elegans is the *Ptilota sericea* of the Phycologia Britannica, and resembles *Ptilota plumosa* so much that, as Harvey tells us, it was at first often considered a shallow-water form of that species. With closer observation it was soon found to be a distinct species, and it has now gone into a distinct genus.

The procarps of *Plumaria elegans* have been described by Bornet ('76). He considered them to resemble in all essentials the procarps of *Callithamnion elegans*, which he has figured. On account of this similarity he proposed to rename the latter plant *Ptilota Schousboei*. For this plant, however, Agardh ('92) has thought it desirable to found a new genus, *Gymnothamnion*. The fact remains, that Bornet's figures of *Callithamnion elegans* were regarded by him as illustrating in all essentials the case of *Plumaria*.

Very recently Davis ('96*c*) has given a full description of the development of the cystocarp in three American plants: *Ptilota serrata*, Kütz (an Atlantic species); *Ptilota plumosa*, C. Ag., and Farlow's variety *filicina* of *Ptilota plumosa*, C. Ag. (both Pacific species). These species are represented by the author as being greatly divergent in the morphology of the procarpial branches from any other species of Ceramiaceae, and he founds upon these peculiarities a theoretical superstructure of great interest.

My own investigations upon the development of the favellae in *Ptilota plumosa* and *Plumaria elegans* lead me, however, to put quite a different interpretation upon the morphological characters they present, and to dissent from the theoretical conclusions advanced by Davis.

The procarpial branches of *Ptilota plumosa* and *Plumaria elegans* are, as in the American species also, smaller pinnae alternating with larger vegetative pinnae. Upon these the favellae arise in an apparently terminal position, surrounded by an involucrate whorl of sterile branches.

The penultimate cell of the procarpial branch may be seen to bulge outwards, i.e. away from the axis upon which the procarpial branch is borne. A cell is soon cut off on this side, and the apical cell is deflected inwards. From these three cells, the apical cell, the sub-apical cell, and the peripheral cell derived from this sub-apical cell, there now arises a group of five branches, each of which ends off in a trichogyne-like process. Of these branches one is terminal, and is more an axial structure than a branch, being a direct continuation of the apical cell: including the cell from which it is derived, it is invariably a 3-celled structure. The sub-apical cell gives rise to two branches one above and one below, each of them being 3-celled. The peripheral cell also gives rise externally to two filaments, one 4-celled and one 3-celled. The order in which they have been now enumerated is not, however, the order in which they arise. The first to arise, it is agreed, is the 4-celled external branch. The carpogenous branch is also derived from the same peripheral cell from which originate the two external branches.

All these five filaments, the terminal, two lateral, and two external, are considered by Davis to be equally carpogonial branches. Before the publication of this view, I had already in the summer of 1896 examined *Ptilota plumosa*, and had come to the conclusion that only one of them is a true carpogonial branch, the other four being mere vegetative filaments terminating in hairs. To the latter I had already given in my notes the name 'pseudo-trichophores.' Upon reading Davis's very clear description, I again examined the procarps of *Ptilota plumosa*, and extended my observations to *Plumaria elegans*, with the result that my earlier opinion was confirmed.

The 4-celled external carpogonial branch is, according to my view, the only true procarp present, the other four being vegetative structures. My reasons for holding this view I submit below.

1. In the first place, this external 4-celled branch has greater morphological fixity than any other branch in the group. It is the first formed, and the only one which is invariably present. Davis alludes to the occasional suppression of the second external branch and of one or both of the lateral branches. In *Plumaria elegans*, which I have found to correspond closely with *Ptilota plumosa* in the general morphology of its procarpal branch, all the filaments other than the 4-celled external branch may remain at times undeveloped, and it is rare to find the whole five ever

developed together. The 4-celled external branch is, however, always present and always complete. Then again, while the number of cells in other filaments varies, though the number is usually three, the branch in question is invariably 4-celled. In *Plumaria*, moreover, I have found the cells of other branches occasionally bud out peripheral cells, therein indicating their vegetative character (Pl. XVIII, Fig. 17). This never occurs in the more highly specialized cells of the true procarp.

2. This 4-celled external branch is the only one which comports itself in its staining and other properties like the carpogonial branch in other Florideae. Treated with Hoffmann's blue, this branch stains more readily, and retains the stain more tenaciously, than any other branch in the group. Indeed, little distinction can be drawn between the behaviour of the other branches and those making up the involucre. All young cells, it is true, stain deeply with this re-agent, but the cells of the true carpogonial branch retain this capacity as long as they can be distinguished. Again, treated with glycerine, the cells of the true procarp swell up much more than those of other branches, a circumstance which may be seen even in Davis's figures, where, however, the gelatinization of the walls is very general. The true trichogyne, too, is more gelatinous than the terminal hairs of other branches. In the latter I have often seen a bend with a kink, like that which appears in an india-rubber tube which is sharply bent.

3. Thirdly, this 4-celled branch corresponds in form and juxtaposition to the carpogenous cell of the procarps of other Ceramiaceae. All Davis's figures show faithfully a crescentic curvature in this branch, which permits it to be readily distinguished. Other branches are straight, or only slightly curved; this is so sharply bent as often to bring the carpogonium back to the mother-cell of the branch, and always sufficiently far to bring it into close contiguity with the carpogenous (auxiliary) cell derived from it. When Davis says that in *Ptilota* the trichogyne (a term in which he includes both the trichogyne proper and the carpogonium

of Schmitz) is 'so far removed from the carpogenous cell that fusion would hardly be possible except through the agency of an ooblastema-filament,' presumably meaning one of some considerable length, he is surely thinking of the remoteness of the supposed trichogynes of other branches. As I have already said, between the carpogonium of the true procarp and the carpogenous cell there is only the separation of the common cell-wall.

Starting upon these two assumptions, (1) that all the branches bearing trichogyne-like hairs are true procarps, and (2) that all the carpogonia are equally remote from the carpogenous cell, both of which I cannot but regard as erroneous, Davis contends that the evidence favours the view that the favellae of *Ptilota* are produced apogamously. In former papers ('96 *a* and '96 *b*) Davis has expressed doubts upon the occurrence of a sexual process in *Batrachospermum* and *Champia*, and it would now seem that he is disposed to include many other Florideae in the same category. Into the discussion of the general question I do not propose to follow him further than to say that apogamy may yet be found to occur in Florideae, as in other groups of plants, but that there is no evidence in favour of the theory that it occurs in *Ptilota*.

In further support of this view, however, Davis refers to the fact that no antheridial plants of *Ptilota serrata* or of *Ptilota plumosa* had been reported. For the antheridia of the Atlantic species he made a determined search, but without success. Upon reading this I decided to overhaul my preserved material of *Ptilota plumosa*, which had been collected however for the sake of procarp-bearing plants. I had no difficulty in finding several fine antheridial plants which had been included unintentionally. The antheridia cover the tips of the branches, and correspond closely in appearance to the similar structures in *Plumaria* which have been figured by Buffham ('93). As far therefore, as the *Ptilota plumosa* of British waters is concerned, the theory of apogamy receives no support from the absence or infrequency of male plants.

I have also repeatedly found spermatia attached to the trichogyne, i. e. the true trichogyne, never to one of the trichogyne-like hairs. Between the carpogonium and the auxiliary cell, moreover, I am convinced that conjugation takes place by means of an ooblastema-tube. I have seen evidences of the existence of the tube, and have shown these preparations to others.

With reference to the passage in the later writings of Schmitz ('92) upon the process of conjugation in *Callithamnion*, though the account is brief and unaccompanied by figures, Davis is mistaken in supposing that it is wanting in precision. Schmitz says, not that he is 'inclined to believe that ooblastema-filaments or their equivalent exist in *Callithamnion*,' but that he obtained similar evidence of a real conjugation in Ceramiaceae, to that which had previously been obtained from *Dudresnaya*, *Polyides*, *Gloiosiphonia*, *Petrocelis*, and other genera of Cryptonemiaceae. From my own observations I may add that I have seen this conjugation in several species, and I have certainly never found in any species of Rhodymeniales any procarp in which the carpogonium and auxiliary cell were so situated that conjugation could not take place between them by means of a short tube.

To revert now to the comparison of *Ptilota* and *Plumaria* with other Ceramiaceae, it is clear that they correspond closely in the essentials of their procarpial structure to the primitive condition displayed in *Antithamnion*. The presence of a number of sterile filaments in the neighbourhood of the procarp usually terminating in hairs, as well as the presence of an involucre, show a considerable amount of specialization and departure from the simpler type.

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THURET: See Bornet et Thuret.

EXPLANATION OF FIGURES IN PLATES XVII AND XVIII.

Illustrating Prof. Phillips' paper on the Cystocarp in Rhodymeniales.

Abbreviations: *aux. c.*, auxiliary cell; *gon. bl.*, gonimoblast-filaments; *st. br.*, sterile branch; *ps. tr.*, pseudo-trichophores; *tr.*, trichogyne.

The material was usually fixed in formaldehyde, and after staining in Hoffmann's blue, mounted in strong glycerine. The cells shaded are those of the carpogonial branch; the red cells are sterile derivatives of the mother-cell of the carpogonial branch. The more heavily defined cells are those of the gonimoblast-filaments.

PLATE XVII.

Figs. 1, 2, 3. Median views of three stages in the development of the cystocarp in *Bonnemaisonia asparagoides*.

Figs. 4, 5, 6, 7. Median views of four stages in the development of the cystocarp in *Plocamium coccineum*.

Figs. 8, 9, 10. Median views of three stages in the development of the cystocarp in *Calleblepharis ciliata*.

PLATE XVIII.

Fig. 11. Apical portion of a branch of *Antithamnion Plumula*, showing two procarps.

Fig. 12. Later stage, showing conjugation of the carpogonium with the auxiliary cell.

Fig. 13. Fertile branch of *Griffithsia corallina*, showing condition immediately subsequent to fertilization.

Fig. 14. Stage showing conjugation of carpogonium with auxiliary cell, and fusion of cells of the carpogonial branch.

Fig. 15. Later stage, showing remains of conjugation-tube in swollen cell-wall; the gonimoblast-filaments have begun to develop.

Fig. 16. Median section of end of procarp-bearing branch in *Ptilota plumosa*.

Fig. 17. Median section of end of procarp-bearing branch in *Plumaria elegans*. The pseudo-trichophore on the right is budding off vegetative cells. One of the branches of the involucre has become a pseudo-trichophore.

Fig. 18. Later stage, showing contiguity of carpogonium and auxiliary cell in *Ptilota plumosa*.

Fig. 19. Shows the two carpogonial branches in *Ceramium tenuissimum*. In one case conjugation of carpogonium and auxiliary cell is taking place.

Fig. 20. Young procarp of *Callithamnion granulatum*.

Fig. 21. Later stage of *Callithamnion byssoides*. The carpogonium has grown out into two gonimoblast-cells right and left, which are closely opposed to the auxiliary cells.

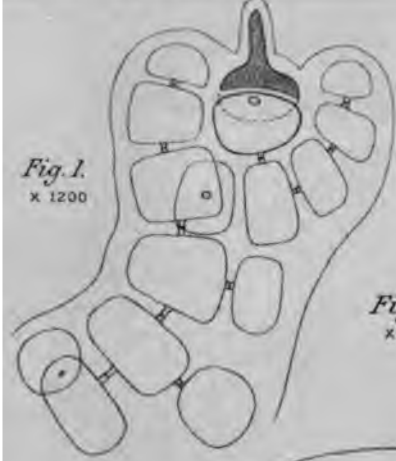

Fig. 1.
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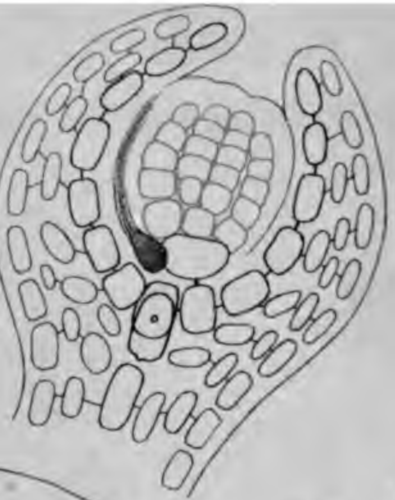

Fig. 2.
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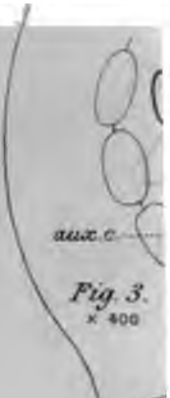

Fig. 3.
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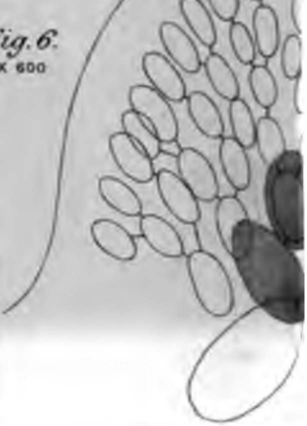

Fig. 6.
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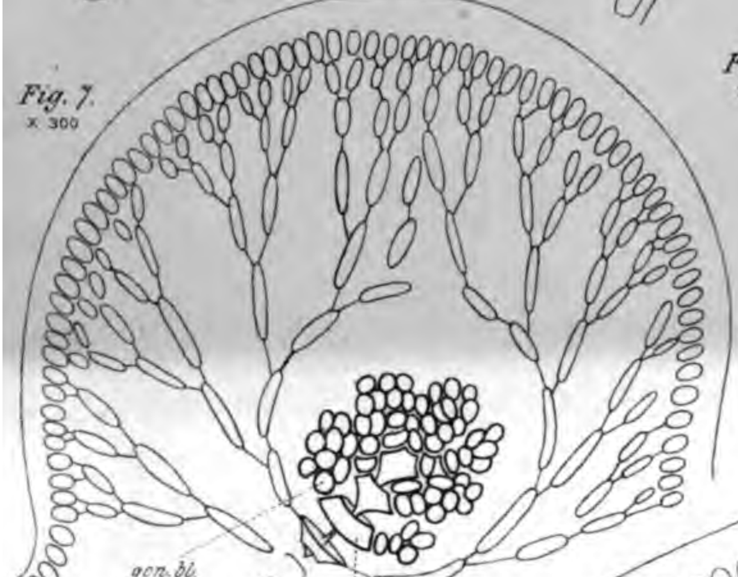

Fig. 7.
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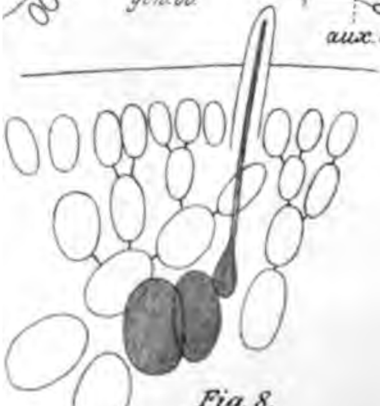

Fig. 8.
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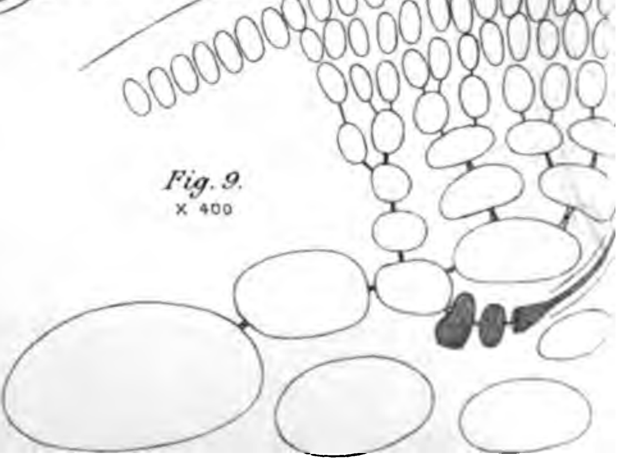

Fig. 9.
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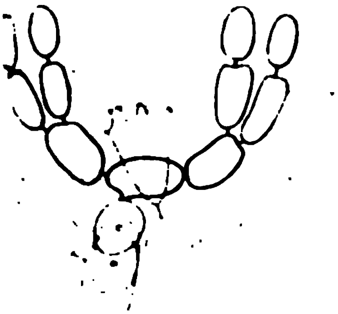
PHILLIPS.—CYSTOCARP IN RHODYMENIALES.



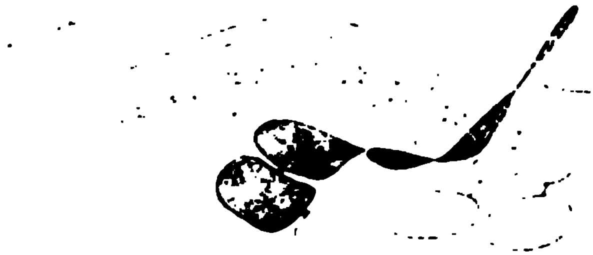


Fig 4
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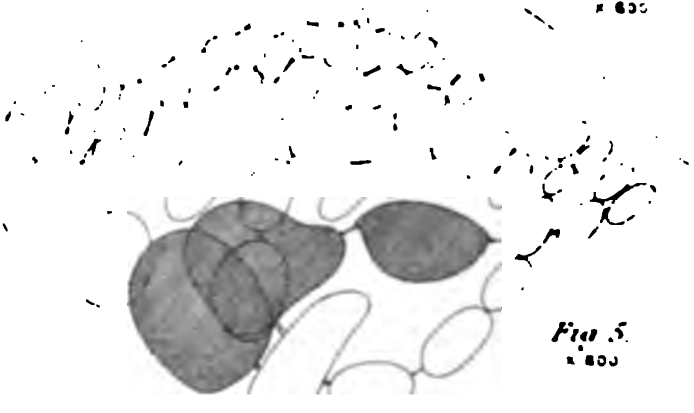

Fig 5
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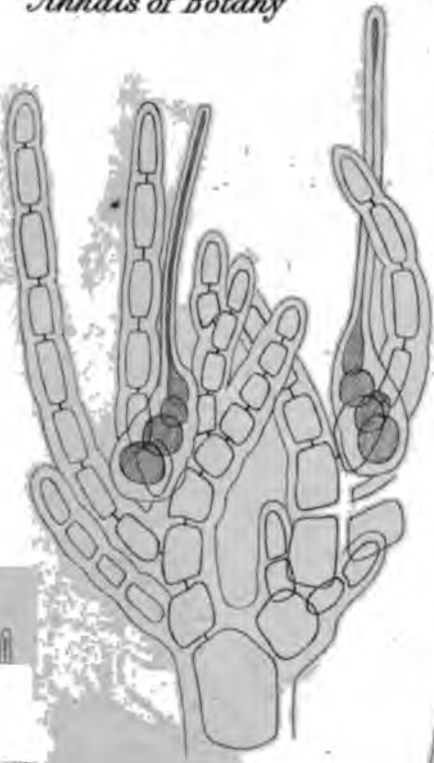

Fig. 11.
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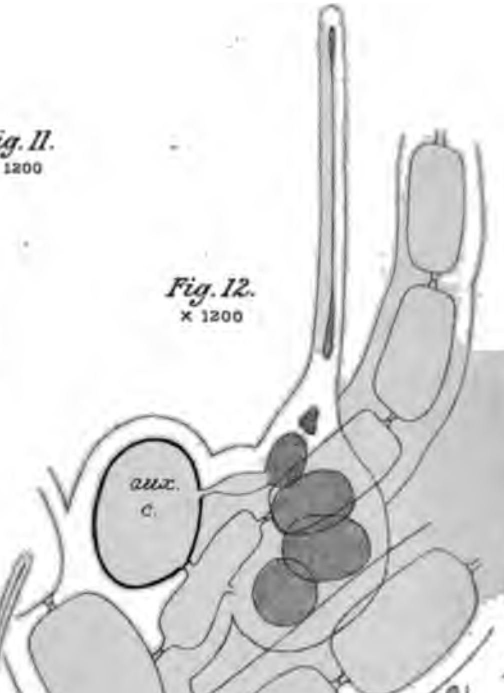

Fig. 12.
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Fig. 1
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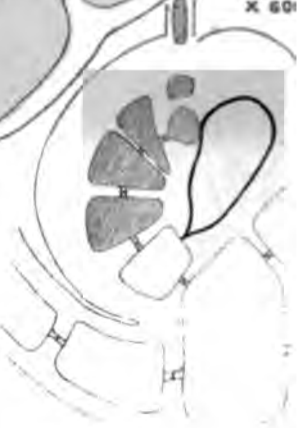

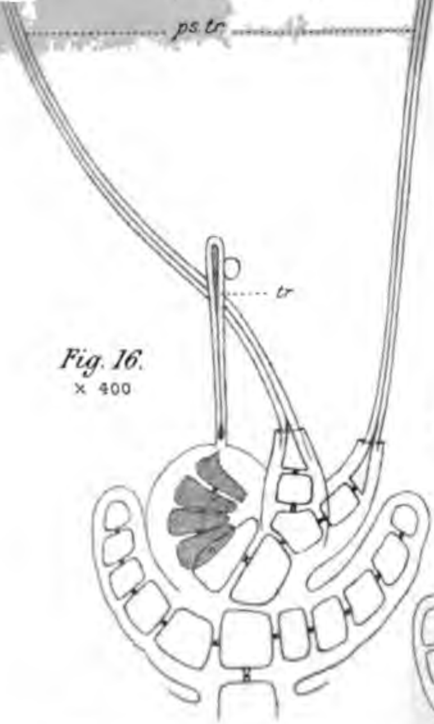

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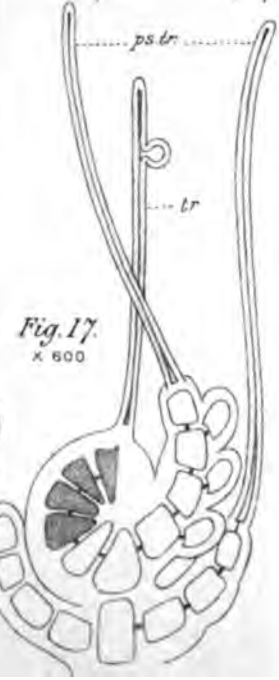

Fig. 17.
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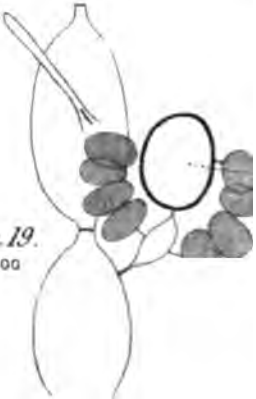

Fig. 19.
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PHILLIPS.— CYSTOCARP IN RHODYMENIALES.

Fig. 13

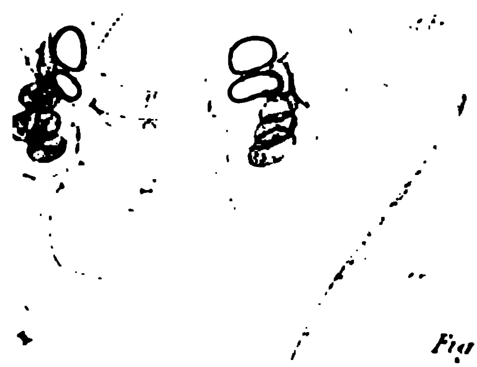


Fig. 14

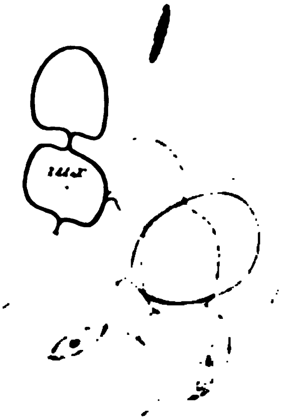


Fig. 15

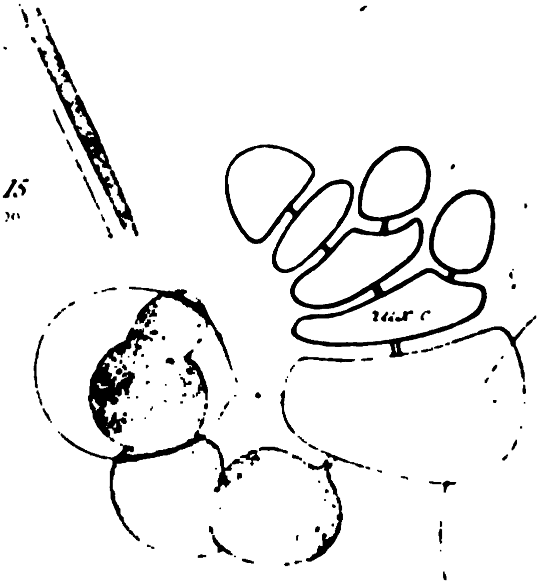


Fig. 20

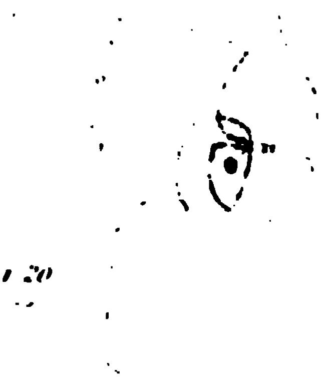
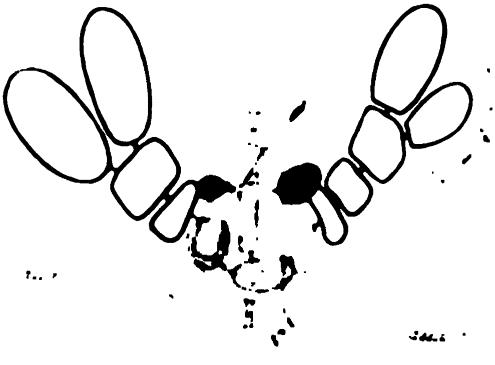
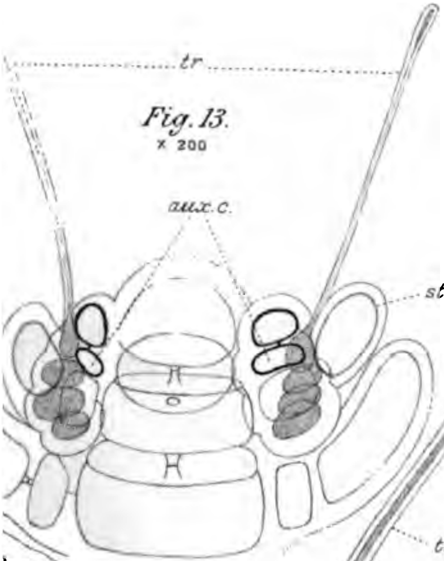


Fig. 21





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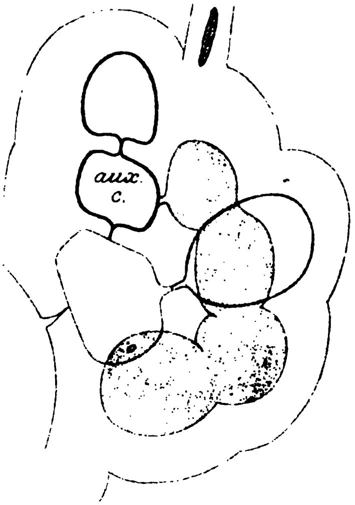

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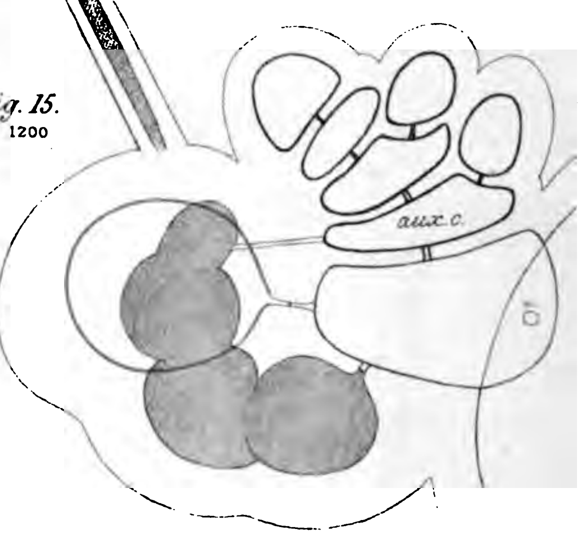

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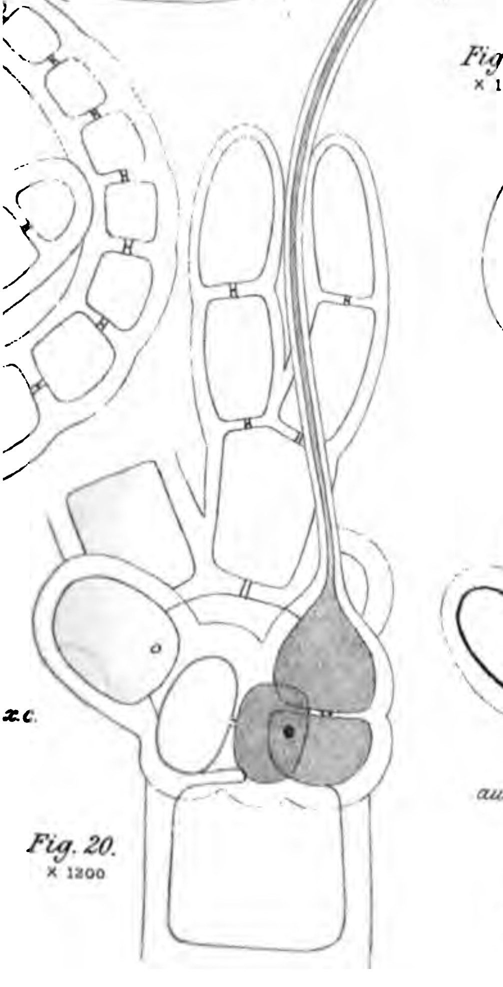

Fig. 20.
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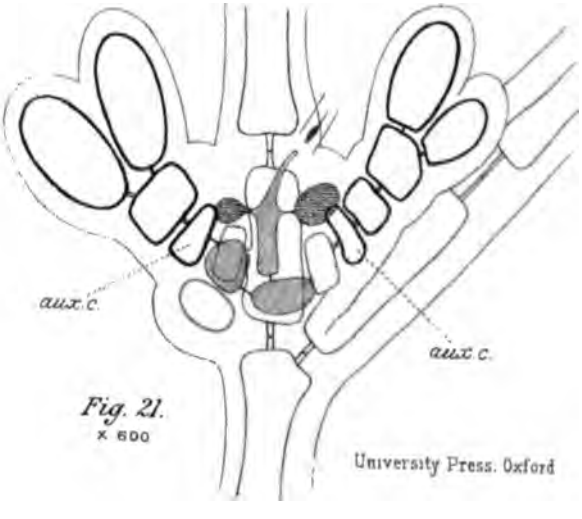

Fig. 21.
× 600

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Obolaria virginica L.: A Morphological and Anatomical Study.

BY

THEO. HOLM.

With Plate XIX and Woodcut 6.

FEW plants, as regards both habit and relationship, have puzzled botanists more than the one known as *Obolaria virginica*, and a comparison of the various diagnoses which have been made of it, even up to a recent date, shows that it is as yet generally very imperfectly known. To thoroughly understand this plant it is necessary to study it in a living state on the very spot where it grows. The many and diverse opinions expressed in regard to its true nature arise from a study of the dried material only.

The name itself has quite a history. The plant which is now dedicated to Linnaeus as *Linnaea borealis* was the first one to be called *Obolaria*, this name being applied to it by Siegesbeck. Gronovius, or rather Linnaeus himself, however, changed the name of this plant to *Linnaea borealis*, and transferred to our North American gentianaceous plant Siegesbeck's name, *Obolaria*, which it still bears. The history of this plant, however, is much older than its present name, extending back to the days of Plukenet and Morison. The former called it *Orobanche virginiana*, and in Morison's and Ray's works it is known under the same name. Later it was described by Clayton, but under no special name, it being

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spoken of as *Anonymos humilis*, and it was after this that Linnaeus gave it the name it now bears.

The *Schultzia obolarioides* of Rafinesque does not seem to be identical with this plant, judging from a comment in the author's diagnosis, in which he says, 'It is very near akin to *Obolaria*'; and also from the fact that, according to his description, the calyx and corolla do not correspond with these organs in our plant. Notwithstanding these differences, however, Otto Kunze adopted the name given by Rafinesque, changing *obolarioides* to *virginica*, O. K.

Considered from a systematic standpoint, there is a great difference of opinion in regard to the classification of this plant. For instance, Elliott, Persoon, and Walter placed it under Didynamia, and Meisner under Scrophularineae; Endlicher believed it to be related to these families; Bartling, Clayton, Don, Jussieu, Lindley, Morison, Plukenet, Rafinesque, and Ray placed it under Orobancheae, while Linnaeus and Willdenow alone considered it as *Orobancheis affinis*. Credit is due to Nuttall for classifying it under the Gentianeae, and to Darlington for having observed that the stamens are equal and not didynamous.

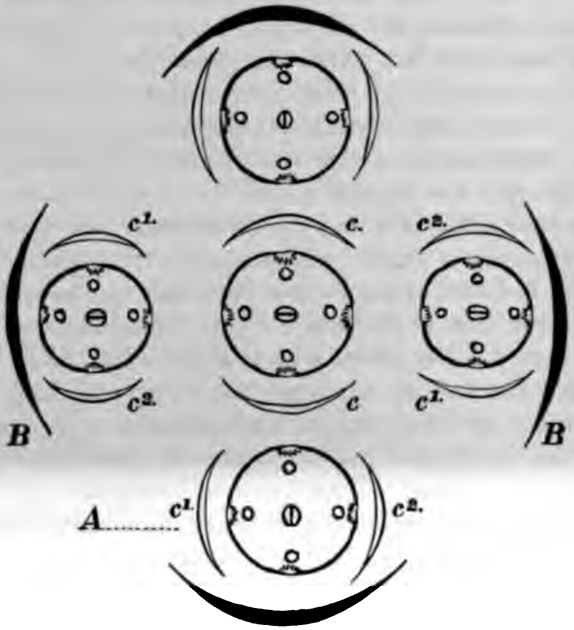
Although the true systematic position of the plant has been ascertained, there still remain several points to be settled. The peculiar aspect of the plant, especially when dried, has led botanists to suspect that it is a saprophyte or even a parasite. Gray speaks of it as 'suspected as partially parasitic'; Johow says it is 'an almost chlorophyll-free humus plant'; Knoblauch believes it to be 'an imperfect saprophyte'; Gilg thinks it is 'doubtless a saprophyte'; and Don has declared that it is 'terrestrial, not parasitic.'

These statements, together with the diversity of opinion in regard to the supposed relationship to the Orobancheae, as indicated above, show the general lack of knowledge in regard to the true nature of the plant.

Notwithstanding the fact that elaborate studies of the *Obolaria virginica* have been published by Baillon, Gilg, Gray, and Knoblauch, there is as yet no detailed description extant, and it is with the hope of adding to the knowledge of this plant and of encouraging botanists to find additional facts in regard to it that we venture to give here some data concerning it.

Various descriptions of the inflorescence of *Obolaria virginica* have been given. Gray first described it as centrifugal, but later described it as a raceme, with the flowers 'in clusters of three, terminal and axillary or solitary'; Bentham and Hooker considered it a spike. However, the fact is that the flowers are arranged in three, or, by abortion, one-flowered cymes. Often small specimens show only a single flower, which is terminal, and at the sides of which rudiments of two lateral flowers are usually found. In large specimens the number of normally developed flowers may reach twenty-one, with rudiments of twelve others, thus corresponding to one terminal and ten lateral three-flowered, or, by abortion, one-flowered cymes. This is a cymose inflorescence, which, according to Eichler, is common in the Gentianeae.

Usually the flowers are sessile, and the peduncles of the inflorescence short, excepting in the case of the lower ones. On examining the flowers two free calyx-leaves will be seen, and this singular fact has given rise to the supposition that *Obolaria* has no calyx, but two bracts instead. Linnaeus considered them a pair of bracts, while Elliott stated that they probably perform the function of a calyx. Even Baillon in his monograph of the Gentianeae described the flowers of *Obolaria* as 'flores asepali.' It is true that the calyx of *Obolaria* is very different from that of the ordinary Gentianeae, not only on account of the suppression of the two leaves, but also because those which are developed are, with the exception that they are smaller, similar to the stem-leaves and the bracts, having the same spatulate shape, with rounded apex, and exactly the same nervation. Their lateral margins are slightly involute, forming an envelope around the flowers, thus performing the true function of an ordinary calyx. Owing to the fact that the position of the calyx leaves in the one-flowered inflorescence corresponds to that of true prophylla, being situated at the right and left sides of the flower, they might at first glance appear to be two prophylla. In the woodcut the single flower *A* shows this arrangement very clearly. If the median three-flowered inflorescence in the same figure is observed, it will be seen that this arrangement does not occur in the central flower. In this flower the leaves (*c* and *c*) are



Woodcut 6.

situated in the front and back of the flower, and not at the sides. The two bracts (*B*) might be considered as the prophylla of the median flower, their place being to the right and left of it. The difficulty in this, however, is that if c^1 and c^2 are the prophylla of the lateral flowers, the median would have not only two prophylla, but also two calyx-leaves. It will be seen, therefore, that this conclusion would not be natural, and it must necessarily be supposed that the flower of *Obolaria* has no prophylla, but only a two-leaved calyx.

In regard to the corolla, it may be said that this is regular, campanulate, and deeply four-lobed, as described by the various authors. In some specimens, however, we have noticed a five-lobed corolla. Notwithstanding this, these flowers had only two calyx-leaves, and the corolla was distinctly bilabiate, with the lower lip three-lobed. In such flowers there were but four normally developed stamens, with a rudiment of a fifth, corresponding to the supernumerary fifth corolla-lobe. The fact that some botanists have been led to believe that the plant is Orobanchaceous may be due to their having observed these abnormal flowers, and also from the singular foliage in connexion with the coralloid root-system.

Another point of interest which so far seems to have been overlooked, is that the corolla of *Obolaria* bears nectaries. When the corolla-tube of a living specimen is opened, there is distinctly visible at the base of each stamen, almost midway between the base of the filament and the base of the corolla-tube, a small, lobate, papilliferous scale (Plate XIX, Fig. 1). As this scale was found in all the flowers we have so far examined, it doubtless is a constant character.

Gilg has given a number of figures illustrating the nectary in the genus *Sweertia*. There is a great similarity between the nectary of *S. multicaulis*, Don, and that of *Obolaria virginica*, and therefore Gray is wrong in saying that the corolla of *Obolaria* has no appendages. Baillon's statement, 'les fossettes disparaissent dans les Bartonias et les Obolarias,' is, on the other hand, nearly correct, since the grooves in the corolla of *Obolaria* are very imperfect. Bentham and Hooker have placed our plant among the Sweertieae, with 'corolla efoveolata'; Gilg has separated our genus from the Sweertieae and their relations, and Knoblauch has followed Bentham and Hooker. The indications are, however, that *Obolaria* is closely related to the genus *Sweertia*.

Another fact which has not been previously noticed is that glandular hairs (Pl. XIX, Fig. 6) are present in the sinuses of the corolla. Baillon speaks of the presence of such hairs in the axils of the bracteoles (calyx leaves), and Knoblauch observed the same not only in the axils of the calyx-leaves, but also in those of the bracts and stem-leaves.

In *Obolaria* the stamens are equal in length. The anthers were figured by Gray as sagittate, but they are not strictly so; and the pollen-grains, which he described as 'globose and very smooth,' are oblong, with the exine distinctly granulate. The ovary is one-celled, and contains a large number of ovules, which are scattered all over the interior surface of the two carpels. In this respect it differs from the other Gentianaceae, with the exception of *Bartonia* and *Pleurogyne*; and as this last-named genus belongs to the Sweertieae, this is another indication that our plant is related to this group. The ovules are anatropous and monochlamydeous, and the funiculus is rather long. Occasionally, however, we have observed that some rudimentary ovules were atropous, and seemed destitute of any integument, thus resembling those of the genus *Voyria*.

The foliage shows a marked uniformity as regards shape and structure. The leaves are opposite, excepting those at the base of the stem, which are sometimes alternate. This, however, is usually due to an occasionally forced curvature of the stem while protruding from the soil. The shape of the leaves is spatulate, this form predominating in the stem-leaves, the bracts, and even in the calyx-leaves. At the base of the stem they are sometimes more or less scale-like. The position of the leaves is somewhat singular, standing in a vertical position and being often closely appressed to the stem and flowers, in which latter respect this plant shows a certain resemblance to the Orobancheae. The colour of the leaves is also characteristic, being of a deep purple hue, with only a slight shade of green. As we have before stated, several writers have expressed the idea that *Obolaria* may be partly or entirely destitute of chlorophyll. The colouration is due to the large amount of anthocyan dissolved in the cell-sap of the epidermis and present in the greater part of that tissue. In this way the chlorophyll is almost entirely concealed, since only the mesophyll is chlorophyll-bearing. By immersing the leaves in strong alcohol, the anthocyan loses its colour, while the chlorophyll stains the alcohol a rich green. It may be added incidentally that it was by this same method that chlorophyll was discovered in the brownish-coloured *Neottia*, which for a long time was believed to be without chlorophyll.

The anatomy of the leaf also shows some points of interest. The cuticula is rather thick and wrinkled, and the cell-walls of the epidermis are strongly undulate, except where the strata cover the nerves, at which points they are almost straight. Stomata are present on both faces of the leaf, but are most numerous on the inferior face. Each stoma is surrounded by four or five cells, and is generally parallel with the longitudinal axis of the leaf (Pl. XIX, Fig. 2). We have observed, however, that some of the stomata, especially in the neighbourhood of the nerves, showed a somewhat different direction, this being due to the stretching of the surrounding cells (Pl. XIX Figs. 3 and 4).

A fact worthy of notice is that the epidermis is but slightly coherent with the parts subjacent, this being observed not only in the leaves, but also in the stem and, in a very marked degree, in the ovary. An examination of the mesophyll shows that it contains the chlorophyll equally distributed in the various layers. A transverse section (Pl. XIX, Fig. 5) shows that the mesophyll consists of about seven layers of roundish cells, and, strange to say, this tissue does not show any differentiation into a palisade or pneumatic tissue. The leaf thus becomes isolateral. Although the isolateral leaves usually have a palisade-tissue developed on both faces of the leaf-blade, a few plants are known in which this tissue is entirely absent. This has, for instance, been recorded by Heinricher as characteristic of the parasitic genus *Thesium*, and it is somewhat surprising to observe a corresponding structure in our genus *Obolaria*.

The absence of palisade-tissue in *Thesium* has been explained by Heinricher as evidently depending upon its parasitic nature, but this explanation does not seem tenable regarding *Obolaria*. A careful examination of the chlorophyll in the leaves shows that it is able to provide its own starch, and this fact seems sufficient to prove that our genus is neither parasitic nor saprophytic. It is also true that the root-system, as will be shown later, does not show any parasitic connexion with the plants which grow in those places where *Obolaria* is generally found.

Concerning the mestome-bundles of the leaves, these are rather weakly developed, and contain only a very small group of leptome and a somewhat larger group of hadrome. Stereomatic tissue was found to be entirely absent, so that altogether the leaf shows a very simple structure.

The stem of *Obolaria* is quadrangular, very pale, and often curved toward the apex. The epidermis consists of rectangular cells, with very thin, straight walls (Pl. XIX, Fig. 7), covered with wrinkled cuticula. Stomata are present (Fig. 8), but they are very scarce. Only a small number of the epidermal cells contain anthocyan. The bark-parenchyma occupies the greater part of the stem-cylinder, and is composed of very thin-walled, loosely-connected cells, with very little chlorophyll and no deposits of starch. No stereome has been observed. Inside the bark-parenchyma is an endodermis (Pl. XIX, Fig. 10); this consists of thin-walled cells, which show the spots named after Caspary, and forms a closed sheath around the mestome-bundles, of which latter the hadrome-element forms the most prominent part. The leptome is developed, not only on the exterior face of the hadrome (Pl. XIX, Figs. 10 and 11), but also on the interior face, toward the pith, and we have in this way what De Bary has designated as 'bicollateral mestome-bundles.' Mestome-bundles of this form have been observed in numerous families, and have been described by various authors, for instance, Moebius and Petersen. According to Gilg, they seem especially characteristic of the Gentianoideae. The exterior leptome is well developed, and forms an uninterrupted ring around the hadrome-cylinder. The interior leptome, on the contrary, forms merely small groups, which border on the inner part of the hadrome. These groups are generally scattered, but a few are connected with each other. It is not unusual, however, to observe some groups of leptome imbedded in the pith (Fig. 12), thus being separated from the hadrome. These isolated leptome-groups are very small, and contain only a few narrow, thin-walled cells. The hadrome is, as stated above, very strongly developed, and the tracheids are so thick-walled and tough that it is difficult to make good sections of the stem so as to get the pith and inner leptome well preserved for study. The pith is composed of thin-walled cells and occupies the entire inner part of the stem.

The root-system has several peculiarities. It consists of a relatively small number of fleshy, irregularly thickened, and wrinkled roots, which show only a few ramifications and are of a light brownish colour. The term 'coralloid,' as used by Morison, for these roots is, therefore, very well chosen. The roots are almost equally developed, there being no main root. In this respect our plant agrees with the Saprophytes, except the European species of *Monotropa*, in which a main root is developed. In our examination of the anatomical structure of the root we were surprised to find that root-hairs are entirely absent. A few of the epidermal cells had become elongated into short papillæ, a fact that again reminds us of the Saprophytes.

An examination of a transverse section of a young root (Pl. XIX, Fig. 13) will reveal a thin-walled epidermis; a hypoderm of only one stratum; a broad bark-parenchyma; and a thin-walled endodermis, inside of which a pericambium surrounds the mestome-bundles. Two groups of leptome, separated from each other by a line of vessels corresponding to two separate groups will also be seen showing that the root is of the diarch type. At this stage the root shows a normal development except that it lacks root hairs. If an older, full grown root (Fig. 14) is examined, it will be found to present a very different aspect. While the structure of the epidermis and the hypoderm agree with that of the young root, the bark-parenchyma will show a great increase in regard to the number of strata and the size of the cells; moreover, the greater part of the bark-cells will be found to be filled with glomerules of fungal hyphae, showing that the root has become a mycorhiza. This form of root was first described by Frank, and, so far, has been found only in a few Gentianaceae, viz. the saprophytic genera *Voyria* and *Voyriella*. According to Warming, mycorhizae are characteristic of several Ericaceae and Orchidaceae which thrive only in a rich humus. In these, as in *Obolaria*, the mycelium is found exclusively in the bark-parenchyma. The mycorhizae also occur in many trees, and Sarauw has given a monographic study of them.

In *Obolaria* the swelling of the roots is due to the presence of these fungal hyphae, which undoubtedly cause a certain irritation, not only of the bark, but also of the tissues inside of the bark. The cells of the endodermis (Figs. 15 and 16) show numerous divisions, both tangential and radial. The pericambium shows similar divisions and forms several strata, while the inner mass of the root-cylinder, the hadrome and leptome, undergoes a considerable disturbance as regards the arrangement of its tissues. The groups of leptome are imbedded in a huge mass of cambial layers, which surround a number of vessels, constituting the innermost part of the root-cylinder. As regards the arrangement of tissues, there is in this a marked difference from that observed in the young roots, as described above.

In the old root there is nothing to indicate its originally diarchic type. The interior tissues, from the bark to the hadrome, undergo an increase so sudden and irregular that the position of the various elements is changed and an abnormal development of cambial tissue takes place. This is all the more peculiar when it is remembered that our plant is annual. Had the anatomical structure of the root been known, there would have been good reason to suspect *Obolaria* to be saprophytic; but as the roots do not show any haustoria there was no reason to suppose it to be a parasite.

In discussing the question as to whether our plant is really saprophytic, as has been supposed, it might be well to cite the principal features which characterize our genus. These may be divided into two categories: (1) Such characters as it has in common with the autophytic Gentianaceae, and (2) the characters by which it is related to the saprophytic members of the Gentianaceous family. In the first category are the following characters: (1) The shape of the aerial leaves is spatulate, not scale-like; (2) the presence of chlorophyll in great abundance; (3) the presence of stomata; (4) the ability to produce starch; (5) the strong development of the hadrome in the stem; (6) the anatropous, monochlamydeous ovules. In the second category, comprising the saprophytic characters, are (1) the vertical, appressed leaves; (2) the non-differentiation of the mesophyll; (3) the imperfect root-system, that is, lack of root-hairs, few ramifications, and non-development of the primary root; (4) the root is a mycorhiza; (5) the occurrence of some rudimentary ovules, these being atropous and destitute of integuments.

According to this characterization, it cannot be denied that our plant shows a singular position between the Autophytes on the one hand and the Saprophytes on the other. The structure of the roots would be rather abnormal to an autophyte, as would be the chlorophyll and the strongly developed hadrome to a saprophyte. In considering the characters of the saprophytic Gentianaceae, so admirably described by Johow, the following differences will be noted. The saprophytic Gentianaceae have no chlorophyll, no stomata, and no lignified tissues, while all these are found in *Obolaria*. The only features, and these are unimportant, which *Obolaria* has in common with its saprophytic relatives are the non-development of the primary root, lack of root-hairs, and the lack of stereome. It grows in soil poor in humus, and in localities which are neither shaded nor very moist, conditions which do not seem especially favourable to Saprophytes. The saprophytic Gentianaceae are all inhabitants of moist and shaded places, being found generally among decayed leaves or on the trunks of dead trees. Being one of our earliest bloomers, its flowers are developed some time before the foliage of the surrounding trees and shrubs unfold, thus being fully exposed to the sunlight, against which it is protected by its waxy cuticula and the large amount of anthocyan contained in the leaves.

The absence of root-hairs in *Obolaria* is of course somewhat surprising in connexion with the non-development of the primary root. On the other hand, however, there are truly saprophytic Orchideae, that is, *Corallorhiza* and *Epipogon*, which have organs that perform the same function as root-hairs, and the European species of the saprophytic genus *Monotropa* possesses a primary root.

On account of the many intergradations between the Autophytes and the Saprophytes, it is almost impossible to draw any general distinctions. In regard to the Gentianaceae, most of which are Autophytes, it may be said that the genus *Obolaria* is a connecting link between the autophytic and the saprophytic genera; and although it has several characters in common with the Saprophytes, we are inclined to consider our plant an autophyte.

In regard to its systematic position, it is, as above stated, closely related to *Sweertia* and *Pleurogyne*: to the first by the presence of nectaries, and to the latter by the position of the ovules on the entire inner surface of the carpels.

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the dividing figures are more or less distorted and obscure. The division of the generative nucleus generally takes place soon after the formation of the pollen-tube[1]. It is already complete in one of my preparations which shows a germinating pollen-grain with a tube of very moderate length (Fig. 34). In this instance the vegetative nucleus looks as though it were in the act of dividing by the direct method, but I have never identified two nuclei at the end of a tube.

Longitudinal fission of the chromosomes is apparent at a comparatively early period in the history of the generative nucleus. It is very clear in the preparation from which Fig. 33 was drawn, and I have seen it unmistakably in six other cases. One of these shows the chromosomes much twisted round each other, and evidently only just formed from the spirem-ribbon. Stages intermediate between those shown in Figs. 33 and 34 are, however, wanting in my preparations. One section which contained a nucleus rather older than that of Fig. 33, the chromosomes being shorter and broader, and the fission in each even clearer, was lost in an attempt to restain it. The absence of such later stages is of the less importance as M. Guignard has figured two in the memoir from which I have quoted so often already (Plate XI, Figs. 33, 34). The stages I possess are conclusive as to the fact of longitudinal fission of the chromosomes.

As we have seen, the pollen-grains are usually brushed on to ridges on the roughened wall of the clefts in the stigma. The pollen-tubes have therefore no great distance to travel in search of them. By the time a tube has reached the nearest cleft, it usually contains three nuclei. The vegetative nucleus is more or less disorganized at the end of the tube. The two generative nuclei are exactly alike, each enclosed in its own envelope of protoplasm (Fig. 34). Neither nucleus possesses a nucleolus or a membrane; they are both in the form of a naked spirem, and they continue thus throughout their journey down the style. M. Guignard has pointed out that they increase in size during this period, and are much compressed in the narrow pollen-tube[1].

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[1] Guignard, l. c., p. 1[illegible].

In describing the three later nuclear divisions of the spermatogenetic series, I have said nothing as to the number of chromosomes which take part in them. There were difficulties in the way of exact counting, but about twelve chromosomes are found on each spindle.

HOMOTYPE AND HETEROTYPE DIVISIONS.

The four nuclear divisions included in the spermatogenetic series of *Lilium Martagon* have now been described in detail. One characteristic—and that the most important—they possess in common with each other, with the three oögenetic divisions, and with the ordinary vegetative division. In each of these cases the effect of the whole process of karyokinesis is to divide each parent chromosome into a pair of daughter-chromosomes by longitudinal fission, and to build up duplicate daughter-nuclei from the duplicate sets of daughter-chromosomes thus formed.

Less uniformity is observed when we come to detail. The seven nuclear divisions of the spermatogenetic and oögenetic series are distinguished from all others by the possession of twelve chromosomes in place of twenty-four. Among these seven divisions thus naturally divided from the rest, two distinct types of karyokinesis are found. One differs from that of the vegetative nucleus only in the number of chromosomes. The other differs widely from the vegetative type in many respects besides that of number, and is characteristic of the first division in either series—that division in which the reduced number of chromosomes first appears.

I have already insisted sufficiently on the peculiar character of the first karyokinesis on either side. The fact that the nuclear divisions, occurring at so critical a period in the line of descent of both sexual nuclei, should not only differ from the usual type, but also resemble each other so closely, is sufficiently remarkable. It must have some meaning When an adequate explanation has been found, it will probably throw light on some of the problems of heredity. In the meantime we may define a difference which our knowledge is insufficient to explain.

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[1] Guignard, l. c., p. 178 and Fig. 36.

The homotype process of karyokinesis is illustrated by the series of diagrams A–F on Plate XI. This is an ideal series, representing what might be expected of an imaginary nucleus, which should divide like a vegetative nucleus (I, p. 451 and Figs. 1–8) while possessing only twelve chromosomes.

Professor Strasburger has described and figured a symmetrical arrangement of the coils in the spirem-form of nuclei from the endosperm of *Fritillaria*[1]. I am convinced that a similar arrangement exists in the much smaller vegetative nuclei of *Lilium Martagon* (B). The chromosomes when they first appear have a ribbon-like character, and retain traces of their late coiled arrangement in the spirem-nucleus (C). They contract and straighten out to a more regular form. Each is bent near one end which lies in the equatorial plane of the spindle. The other points to one of the poles. Longitudinal fission first appears in this stage (D). Later on the whole chromosome comes to lie more or less in the equatorial plane, and separation of the two halves begins at the end which is attached to the spindle (E). The diaster-segments are necessarily hooked (F).

Each of the five homotypic divisions is now to be compared with the ideal series A–F.

(1) The division of the micropylar nucleus in the binucleate embryo-sac agrees with it in all but two points. This nucleus when in the spirem-form, and also the young chromosomes of the succeeding stage, are apt to be pulled out in the direction of the future spindle thus concealing the symmetry of the spirem coils (I, p. 464 and Figs. 28, 29). Besides this deviation from the type there is a second occasional variation. In a few chromosomes of the nuclear plate—one or two

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[1] Strasburger, Ueber Kern- und Zelltheilung, 1888, pp. 61–63, Taf. II, [illegible]

perhaps on each spindle—the segments begin to separate from the middle instead of from one end, and in consequence these segments are V-shaped in the diaster (I, pp. 465–66: × Figs. 30, 31).

(2) In the succeeding division—that of both micropylar nuclei in the four-nucleate embryo-sac—similar deviations from the type occur. The spirem is distorted, and an occasional chromosome begins to divide about the middle of its length. The latter variation, however, is much less common than in the previous division (I, p. 468).

(3) The second division of the pollen-mother-cell nucleus is by far the most aberrant form of the five. The spirem indeed is quite typical (Fig. 16). But the chromosomes are V-shaped from the beginning and regularly divide from the middle. One or two in each spindle may divide from one end. Consequently most of the diaster-segments are V-shaped (Figs. 19 and 21).

(4) The nuclear division in the pollen-grain which divides the generative from the vegetative nucleus is typically homotype. It agrees in every stage with the ideal series (Figs. 24–28).

(5) Those stages in the division of the generative nucleus which I have seen—namely, spirem, segmentation, and early spindle—are certainly homotype. The spirem is compressed in the pollen-tube, but not violently distorted. Longitudinal fission appears rather early in the chromosomes. But the close likeness between Fig. 33 and Diagram D cannot be mistaken.

In describing the heterotype form of karyokinesis there will be no need to begin with an ideal series and afterwards discuss the deviations from it of the two nuclear divisons which follow that type. The diagrams G–M are founded on the first division of the embryo-sac nucleus. If they had been founded on the first division of the pollen-mother-cell nucleus, the size of the chromosomes would be greater in proportion to the spindle and the diameter of the nucleus. In all other respects the diagrams represent either division equally well.

One important feature of heterotype karyokinesis is not represented in the diagrams. No indication is given of the long period of growth which ends in the formation of the spirem (G). The most prominent character of this period is the occurrence within it of the contracted state called synapsis, already twice described in detail. I believe the physiological purpose of this condition to be the formation of the spirem-ribbon from a single row of chromatin-granules strung on a linin-thread. This row is divided longitudinally just before the contraction begins, and during that contraction the linin-ribbon, which separates the two rows of granules in the spirem-nucleus, is formed at the expense of the half-dissolved nucleolus (I, p. 458, and II, p. 198).

The other features which distinguish heterotype from homotype divisions are fairly represented in the diagrams. They may be summarised here.

Differentiation of the spirem-ribbon. In Diagram G, as in A, the spirem-nucleus contains a coiled chromatic ribbon and one or more nucleoli within the nuclear membrane. But in A the ribbon appears to be homogeneous—its whole surface reacts in a uniform way to stains; and in G it is differentiated. The ribbon itself is erythrophilous, but it bears on either margin a row of cyanophilous granules. There may even be some further differentiation. At certain periods in the development of the ribbon, it seems as if the borders on which the granules lie were of rather tougher material than the broad zone which separates them.

Early longitudinal fission of the chromosomes. In Diagram C the homotype chromosomes are seen to be formed of lengths of spirem-ribbon. Longitudinal fission follows later (D). In the heterotype nucleus the spirem-ribbon is first divided longitudinally and then falls into lengths. Thus each length consists of two separate linin filaments, each bearing a single row of chromatin granules (H). The nuclear membrane and nucleoli disappear before segmentation in the homotype nucleus (B), but persist for some time later in the heterotype (I).

Second longitudinal fission of the chromosomes. The segments of homotype chromosomes appear homogeneous throughout the karyokinesis (D–F). Heterotype segments are differentiated shortly after their formation. Each consists of a length of erythrophilous ribbon bearing a row of cyanophilous dots on either margin (I). This differentiation disappears with the nucleolus and nuclear membrane. The segments then look homogeneous (K). Whether any trace of the second fission remains in their structure is uncertain.

Contorted shape of the chromosomes in the nuclear plate. The segments of the homotype chromosome are nearly parallel to each other, and they separate from each other with great regularity, usually from one end (E). This is not the case with the heterotype chromosome. The segments are tightly twisted on each other, they may separate from near the middle of the chromosome or (more rarely) from one end. The result is that the separating chromosomes are much contorted, and adjacent ones may be of a very different shape (K).

Shape of diaster-segments. As a necessary consequence of their method of separation, segments of homotype diaster are generally hooked (F), the heterotype diaster-segments V-shaped (M). No great importance is to be attached to this distinction.

APPENDIX ON METHODS.

A. FIXING.

Anthers fixed in absolute alcohol were usually uncut. Those fixed in any of the three solutions given below were either halved transversely or cut at both ends to ensure penetration.

Hermann's solution (alcoholic).

10 % aqueous solution of platinic chloride	3 c.c.
1 % osmic acid (aqueous) . . .	8 c.c.
Glacial acetic acid	2 c.c.
Absolute alcohol	27 c.c.

The anthers were left in this solution for one and a half to two hours, and then transferred to a ·5 % aqueous solution of platinic chloride for twenty-four hours. They were then placed in a 1 % aqueous solution of platinic chloride for twenty-four hours.

Flemming's solution (*aqueous*).

1 % aqueous solution of chromic acid	30 c.c.
1 % osmic acid (aqueous)	8 c.c.
Glacial acetic acid	2 c.c.

The anthers were left in this solution for about two hours, and then transferred to an aqueous ·5 % solution of chromic acid for eighteen hours.

Chromic acid (*aqueous*). The anthers were laid in a ·5 % aqueous solution of chromic acid for eighteen to twenty-four hours.

After treatment in any of these ways, the anthers were rinsed in water, and transferred successively at intervals of about twelve hours to 30 %, 50 %, 70 % alcohol, and finally left for several days in methylated spirit, changed as it became discoloured. These changes were made in the dark when the fixing solution had contained chromic acid.

The anthers were preserved in a mixture of equal parts absolute alcohol, glycerin, and distilled water.

B. EMBEDDING AND CUTTING.

For anthers fixed in mixtures containing chromic acid or platinic chloride, paraffin melting at 52° C. is hard enough. A softer paraffin can be used for anthers fixed in absolute alcohol. I was able to cut sections 15 μ thick from such material embedded in paraffin melting at 45° C.

The sections were usually floated on the slide with distilled water, and made to adhere by careful drying without cement. But in the case of anthers showing the nuclear division within the nearly mature pollen-grain, I used a cement of collodion and clove-oil, otherwise the sections of pollen-grains would have been washed away.

Great care must always be taken not to overheat the paraffin-ribbon on the slide. If the paraffin approaches its melting-point, the sections will be strained and their structure distorted.

Hand-sections are apt to be broken while they are being transferred from a stronger to a weaker solution of alcohol. To avoid this the sections were placed in a small wide-necked bottle half filled with distilled water, on the top of which absolute alcohol had been poured gently. The alcohol floated for some time on the water, and the sections sank down through solutions of gradually increasing density until they lay in the pure water at the bottom. Then the alcohol was drawn off by a pipette.

C. STAINING.

1. *Flemming's orange method for material fixed in Flemming's solution, Hermann's solution, or chromic acid.*

There is little to add to the account given in Part I, p. 474. For early stages in the development of the pollen-mother-cell, the potassium permanganate was used as a mordant both before and after the treatment with safranin. The safranin and gentian violet solutions were also of double the usual strength for these stages. For later ones—as the first nuclear division in the pollen-mother-cell—the ordinary treatment was sufficient.

2. *Mayer's haemalum for chromic material.*

The sections were placed for half an hour in a ·5 % solution of ferric chloride in water, rinsed and transferred to Mayer's haemalum, nearly full strength. They usually took about two hours to stain to the right depth. If the sections were kept alkaline by rinsing in hard water and by the use of neutral alcohols, they were of a brilliant blue and very permanent.

3. *Mayer's haemalum for absolute alcohol-material.*

The sections were treated as above, but with ·1 % solution of ferric chloride for half an hour, and 10 % solution of Mayer's haemalum in ·1 % solution of potash alum for about twelve hours.

4. *Methyl-green and acid fuchsin for alcohol-material.*

These colours were used in aqueous solution, their proportions varied to suit different stages.

5. *Renaut's haematoxylic eosin for alcohol-material.*

The same treatment as that given in Part I, p. 474, was used.

POSTSCRIPT.

The twisted V-shape is characteristic of the diaster-segment in both heterotype divisions, and may easily give rise to misconception. Thus Professor E. B. Wilson (The Cell in Development and Inheritance, p. 197) has pointed out that one of my figures, representing a pair of such chromosomes *en face*, would suggest that each daughter-chromosome is already divided transversely. Such an appearance is in fact not uncommon, but in studying the actual preparation it can as a rule be quite clearly made out that the daughter-chromosomes are neither divided nor even constricted. They are usually contorted, however, by a sort of double twist near the middle of each. This may be more easily followed in the chromosomes of the first embryo-sac karyokinesis (I, Figs. 23 and 24. Compare especially the chromosomes 1–4 in Fig. 24 with those of Fig. 23 and with Figs. 13 and 14 of this part). It will be seen later on that this twisted V-shape reappears in most of the chromosomes belonging to the second karyokinesis in the pollen mother-cell (Figs. 18, 19). No transverse division has therefore taken place since the first karyokinesis, nor is any indicated in the chromosomes of the daughter-nuclei, which shortly afterwards divide by longitudinal fission (p. 207).

QUARRY HILL, REIGATE
April 27, 1897.

EXPLANATION OF FIGURES IN PLATES X AND XI.

Illustrating Miss E. Sargant's paper on the Nuclei of *Lilium Martagon*.

The figures marked × 1365 were drawn from Zeiss' apochromatic hom. im. objective, 2 mm. focal length, N.A. 1.30, with eye-piece 4, and those marked × [illegible] and × 1930 with the same objective, eye-piece 8. The figures marked × 1000 were drawn under Zeiss' apochromatic hom. im. objective, 1.5 mm. focal length, eye-piece 6. Figs. [illegible] and 31 were drawn under Zeiss' apochromatic objective, 16 mm. focal length, with eye-pieces 6 and 4 respectively. All these figures were outlined with the camera lucida and details added in freehand.

Fig. 29 was slightly enlarged by eye from a whole stigma.

PLATE X.

Figs. 1–15. *First karyokinesis in pollen-mother-cell.*

Fig. 1. Pollen-mother-cell soon after its formation, with resting-nucleus. × 585.

Fig. 1 *a*. Resting-nucleus from older mother-cell: *n*, nucleolus. At × the thread shows dots. × 1000.

Fig. 2. Pollen-mother-cell with nucleus in which first signs of approaching synapsis are seen. The chromatic network begins to contract away from the nuclear wall to the right of the figure. Besides the vacuolated nucleolus, there is a shapeless mass of nucleolar matter to the left, which is green in the thicker part about ×, but washy red at the edges. × 585.

Fig. 2 *a*. Bit of thread from nucleus of same age as that shown in Fig. 2, more highly magnified. The thread is irregularly thickened, and shows two rows of dots. At *n*, *n*, drops of nucleolar matter are attached to it.

Fig. 3. Pollen-mother-cell with nucleus in synapsis. A general view of the whole cell from a hand-section. The nucleolus is hidden by the close coils of the contracted chromatic thread. × 585.

Fig. 3 *a*. Section from nucleus rather older than that drawn in Fig. 3: *n*, the nucleolus, now recovering its shape. There is still a good deal of amorphous nucleolar matter among the coils of the contracted chromatic thread. × 585.

Fig. 3 *b*. Bit of thread (from place marked × in Fig. 3 *a*), more highly magnified. It is now of uniform breadth and the double row of dots is clear.

Fig. 4. Pollen-mother-cell with spirem-nucleus. From a hand-section. × 585.

Fig. 4 *a*. Tangential section from nucleus rather older than that of Fig. 4. The ribbon begins to be articulated. × 1000.

Fig. 5. Pollen-mother-cell with nucleus in which the spirem-thread has begun to split longitudinally (× ×′): *n*, nucleolus. × 585.

Fig. 5 *a*. Tangential section from nucleus in which the splitting of the ribbon is almost complete. × 1000.

Fig. 6. Pollen-mother-cell in which the spirem-ribbon of the nucleus has begun to fall into lengths, as at ×. Longitudinal fission is already complete: *n*, nucleolus. From the same hand-section as Fig. 5. × 585.

Fig. 7. Pollen-mother-cell with nucleus, in which the twelve double filaments formed from the spirem-ribbon have begun to cluster round the half-dissolved nucleolus. The nuclear membrane is indistinct, and much of the sap is coloured by the nucleolus. × 585.

Fig. 7 *a*. Section of nucleus nearly the same age as that shown in Fig. 7. From a series of thick sections (15 μ) from alcohol-material. All the phenomena of synapsis have reappeared. × 1000.

Fig. 8. Pollen-mother-cell from same section as Fig. 7. The optical section of the nucleus probably passes through the smallest diameter of the nucleus. The filaments are shorter and thicker; probably each consists of a double row of dots, but the section is too thick to make this certain. The nucleolus assumes its definite outline and the nuclear membrane reappears. × 585.

Fig. 8 *a*. Part of a single pair of filaments from a nucleus rather older than that shown in Fig. 8. Each filament is irregularly thickened and bears a double row of dots. Magnified about 1000 times.

Fig. 9. Pollen-mother-cell from a thin serial section. The immature chromosomes consist of two twisted lengths of linin-ribbon, each bearing two rows of

chromatin-granules: *n*, nucleolus, round which some of the chromosomes are still clustered. × 585.

Fig. 9*a*. Single immature chromosome from nucleus about same age as that of Fig. 9, more highly magnified.

Fig. 10. Pollen-mother-cell from thick serial section (15 μ). The chromosomes are nearly mature: *n*, *n*, fragments of nucleolus. × 585.

Fig. 10*a*. Pollen-mother-cell from serial section (10 μ thick). The spindle is just appearing; part of the nuclear membrane is still seen at ×. Small drops, probably of nucleolar matter, are visible in the cytoplasm. × 585.

Fig. 11. Pollen-mother-cell from same section as Fig. 10. The asymmetrical spindle is just formed. × 585.

Fig. 11*a*. Pollen-mother-cell from thick serial section (12·5 μ). The spindle is still asymmetrical, and though the chromosomes are attached to the spindle-fibres, they are not as yet arranged in an equatorial plate. × 585.

Fig. 12. Pollen-mother-cell from thin serial section (7·5 μ). Part of the nuclear plate is seen tangentially. × 585.

Fig. 13. Pollen-mother-cell from thin serial section (10 μ). Four chromosomes are seen just before the separation of their segments is complete. × 585.

Fig. 14. Pollen-mother-cell from same preparation as Fig. 12. The daughter-chromosomes are formed and on their way to the poles. Each is V-shaped, with the angle more or less bent back. × 585.

Fig. 15. Pollen-mother-cell from same anther as Figs. 12 and 14. Two daughter-nuclei are formed with cell-plate between them. × 585.

Figs. 16–22. *Second karyokinesis in pollen-mother-cell.*

Fig. 16. Tangential view of daughter-nucleus in spirem-stage. × 1000.

Fig. 17. Similar view of rather older nucleus. The coils are being pulled out in the direction of the future spindle. × 1000.

Fig. 18. Nine chromosomes formed from the spirem-daughter-nucleus. Nos. 1, 2, 3, 4, 5, and 9 are V-shaped. × 1000.

Fig. 19. Five chromosomes on spindle of daughter-nucleus. All show V-shape. × 1000.

Fig. 21. Two chromosomes from nuclear plate of daughter-nucleus just as segments are separating. × 1000.

PLATE XI.

Fig. 20. Pollen-mother-cell divided into two compartments, each containing a nuclear plate. × 585.

Fig. 22. Pollen-mother-cell divided: the nucleus of each daughter-cell has formed a diaster. × 585.

Figs. 23–28. *Karyokinesis of pollen-grain-nucleus.*

Fig. 23. Sections of immature pollen-grain, (a) parallel to long axis, (b) perpendicular to long axis. *n*, nucleus. × 300.

Fig. 24. Section of pollen-grain with nucleus in which the spirem-ribbon is formed. A differentiated mass of cytoplasm is seen near the nucleus. × 585.

Fig. 25. Tangential view of nucleus in spirem-stage: *n*, nucleolus. × 1000.

Fig. 26. Five chromosomes arranged on spindle. Longitudinal fission not yet apparent. × 1000.

Fig. 27. Four chromosomes on spindle. The segments of each are in the act of separating. × 1000.

Fig. 28. Section of pollen-grain in which the daughter-chromosomes of the dividing nucleus are just separating. At the inner pole is a differentiated mass of cytoplasm. × 585.

Figs. 29–34. *Karyokinesis of generative nucleus in pollen-tube.*

Fig. 29. Stigma slightly enlarged.

Fig. 30. Section of pollen-grain *in situ* on stigma and just about to put out tube: *v. n.* vegetative nucleus; *g. n* generative nucleus already in spirem-stage. × 585.

Fig. 30 *a*. Empty shell of pollen-grain with remains of tube, from dissection of stigma. × 100.

Fig. 31. Stigmatic hairs with pollen-grains germinating among them: *p. t.* tube from which exine (*ex*) has fallen off, and containing generative nucleus (*g. n.*); *p.g.* pollen-grain which has put out coiled tube. × 66.

Fig. 32. Pollen-tube containing generative cell in which generative nucleus is on the eve of forming chromosomes from lengths of spirem-ribbon. × 1000.

Fig. 33. Pollen-tube in which generative nucleus has formed twelve chromosomes, each divided longitudinally. × 1000.

Fig. 34. Germinating pollen-grain from dissection. Three nuclei. × 290.

DIAGRAMS.

The two series of diagrams illustrate the difference between the heterotype process of nuclear division in *Lilium Martagon* and the homotype process.

The homotype series is founded on drawings of the vegetative nuclei which are seen in tissues of the young ovule and ovary (I, p. 451). They are drawn as though each nucleus had twelve chromosomes in place of twenty-four.

The heterotype series is founded on drawings from the first division of the embryo-sac nucleus. The spindle is drawn on the same scale as the homotype spindle, which makes the heterotype chromosomes appear smaller than the homotype chromosomes. For the ratio of spindle-length to chromosome-length is greater in the heterotype than in the homotype karyokinesis.

EXPLANATION OF FIGURES IN PLATE XIX.

Illustrating Mr. Holm's paper on *Obolaria virginica*.

(The letters in the Figures indicate as follows: *B*, Bark-parenchyma; *Ca*, Cambium; *End*, Endodermis; *Ep*, Epidermis; *H*, Hadrome; *Hyp*, Hypoderm; *L*, Leptome; *M*, Mesophyll; *P*, Pith; *P. H*, Protohadrome; *Pr*, Pericambium; *V*, Vessels.)

Fig. 1. One of the papilliferous scales from the corolla. × 65.

Fig. 2. Stoma from the superior face of a stem-leaf. × 440.

Figs. 3 and 4. Two stomata from the inferior face of a stem-leaf; these were taken from that part of the epidermis which covers the veins. × 440.

Fig. 5. Transverse section of a stem-leaf. Some of the epidermal cells contain anthocyan (drawn with dots in the Figure), and the mesophyll forms a uniform tissue without being differentiated into any special palisade or pneumatic tissue. × 440.

Fig. 6. Glandular hair from the axil of one of the stem-leaves. × 440.

Fig. 7. Epidermis of the stem. × 440.

Fig. 8. Stoma from the stem. × 440.

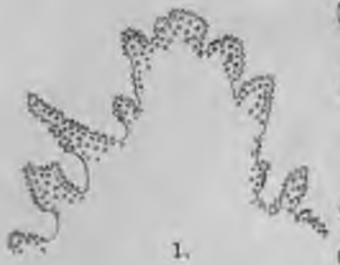
Figs. 9, 10 and 11. Transverse sections of the stem, showing the epidermis and a part of the bark (Fig. 9), the endodermis, a group of leptome, a part of the hadrome (Fig. 10), two groups of the inner leptome, and a part of the pith (Fig. 11). × 440.

Fig. 12. A very small group of the inner leptome imbedded in the pith. × 440.

Fig. 13. Transverse section of a young root, showing a part of the inner layers of the bark, the endodermis, the pericambium, the leptome and hadrome. × 440.

Fig. 14. Transverse section of a full-grown root, showing the inner layers of the bark-parenchyma, of which two cells show glomerules of fungus-hyphae. The endodermis and the pericambium show numerous tangential and radial divisions. Between the leptome and hadrome is a great mass of cambial tissue. × 440.

Figs. 15 and 16. Two cells of the endodermis of a full-grown root showing radial and tangential divisions. × 440.



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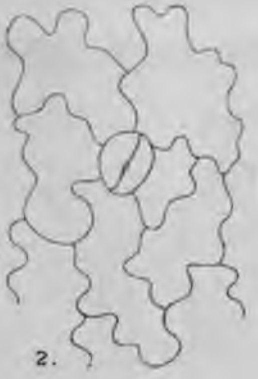

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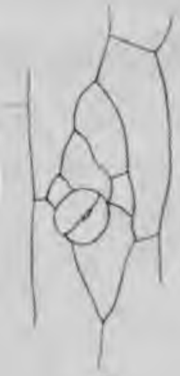

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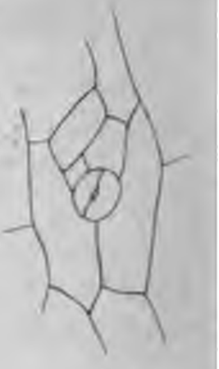

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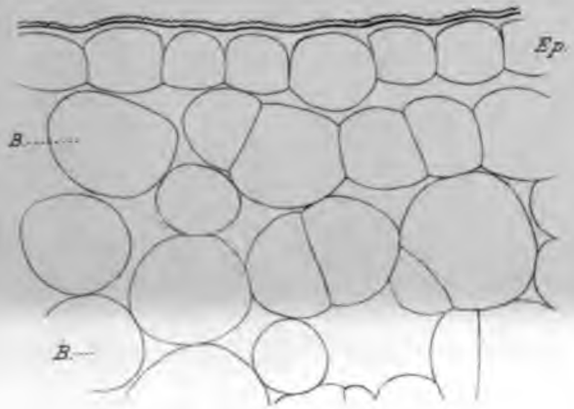

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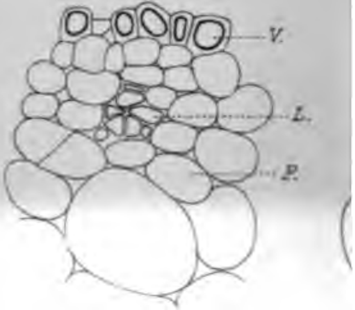

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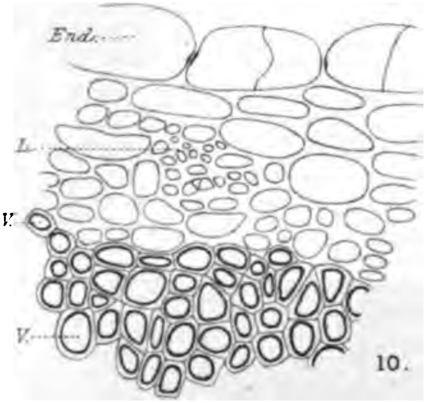

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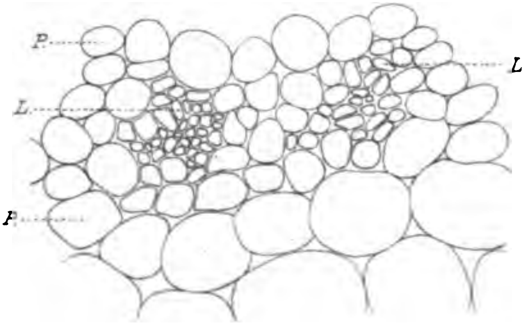
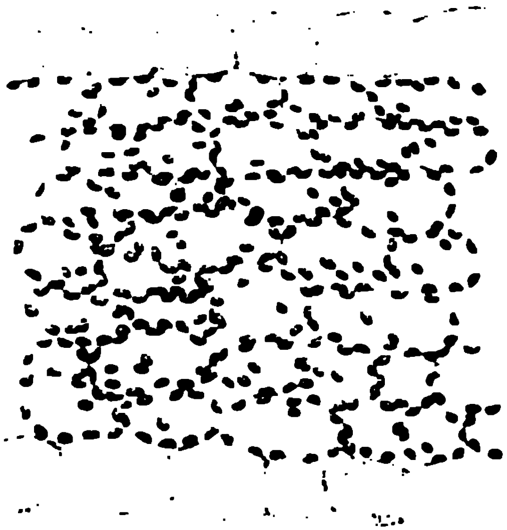


11.

Theo.Holm del.

HOLM. — OBOLARIA VIRGINICA.



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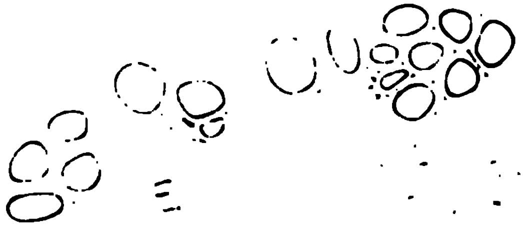

10

On the leaves of Lathraea Squamaria and of some allied Scrophulariaceae.

BY

PERCY GROOM, M.A., F.L.S.

With Woodcut 7.

IN a former paper (8) I have attempted to show that certain saprophytes possess structures which enable them to get rid of any excess of water absorbed during their subterranean period of existence. In particular, I drew attention to the fact that saprophytes may possess stomata or peculiar epidermal patches on their underground shoots, which would facilitate the excretion of water either as liquid or vapour. It seemed probable that if my suggested explanation of these histological details were correct, some corresponding mechanisms might be discovered in other geophilous plants, and even in parasites accustomed to a partially subterranean mode of life.

Lathraea Squamaria suggested itself as a favourable subject for study, particularly as this plant can go through its whole cycle of life completely embedded in the soil; even forming cleistogamic subterranean flowers, and being capable of regenerating itself, when broken, by means of fragments of its

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shoots. Further, this plant is very frequently found in soil which is very moist, or thoroughly wet, during the active vegetative season. It consists of a branched rhizome bearing many lateral roots. The roots spring from the sides of the rhizome and bear many lateral haustoria. The scales on the rhizome are arranged in a decussate manner and closely packed together. Each scale is pocket-like in form, with the mouth of the branched pocket-cavity opening on the lower face of the leaf. The epidermis lining the cavity represents the lower face of the leaf; it is devoid of stomata, but possesses hairs of two sorts:—(1) shortly-stalked capitate hairs; (2) peculiar dome-shaped hairs, often described as sessile glands. Both forms of hairs are glandular in appearance.

Proof of the excretion of water. A healthy flowering specimen of the plant was dug up from damp soil. Water was forced into the open ends of cut shoots by means of columns of mercury varying from 12–24 inches. Subsequent examination, after a lapse of some hours, showed that excretion had been so vigorous as to cause an outflow of water from the pockets of the younger leaves. I could not prove that there had been any excretion of water from the oldest leaves: there was not sufficient to cause an outflow from their pockets which contained both air and liquid. Larger parts of the plant kept, with rhizomes and roots dipping into water, and in a saturated atmosphere, did not excrete water sufficiently rapidly to cause any overflow from the pockets.

Owing to the peculiar form of these leaves, it is impossible to see directly which portions of the epidermis lining the pockets are responsible for the excretion of water: it was only possible to show that the water came from the cavities, and not from the epidermis of the upper face. Experiments of forcing coloured solutions into the leaves gave no satisfactory results. Fortunately a number of parasitic Scrophulariaceae (3) possess hairs like those of *Lathraea*; I therefore examined several of these plants.

PEDICULARIS PALUSTRIS.

Inasmuch as this plant lives in damp, even marshy, spots, it seemed probable that it would possess a water-excreting mechanism. Its much-lobed leaves have their margins recurved downwards. On the lower face of the leaf very numerous dome-shaped glands lie in furrows; over the fine nerves, especially near the margins, the glands are so numerous and large in some spots as to be in lateral contact to the exclusion of general epidermis. The vascular bundles of the leaf end in fine branches running close to the epidermis of the lower face, and therefore very near to dome-shaped glands. These fine nerves are separated from the upper epidermis by the well-developed palisade-parenchyma. The shortly-stalked capitate glands are exceedingly rare on the lower epidermis, but long-stalked hairs occur along the courses of the grosser nerves on that face. Numerous stomata are present on the lower face. Dome-shaped glands and stomata are absent from the upper face of the leaf; but a few capitate hairs occur above the courses of the nerves.

Excretion of water from the leaves. When water was forced up shoots under a pressure of 1 inch of mercury (in addition to the atmospheric pressure), it sufficed to cause an instantaneous excretion of water from the leaves. But when a pressure of several inches of mercury was applied, the water gushed out from the leaves with great rapidity. All the young leaves were dripping with moisture, and water was to be seen falling from the leaf-tips and running down the surface of the stem in swift little streams, also welling up, fountain-like, from the leaf-axils. The very violence of the outpour rendered it impossible to localize its exact source. Inverting an excreting branch served to demonstrate that the young leaves were mainly responsible for the excretion of water, and that in the first experiment the stem merely conducted the water down the hairy lines on its surface, and thus caused the overflow at the leaf-axils.

Histology of the dome-shaped glands. The excessive number of the dome-shaped glands, their close connexion with the tracheides terminating the vascular bundles, the separation of the latter from the upper face of the leaf, and finally the instantaneousness of the outflow, all pointed to the dome-shaped glands as being responsible for the excretion of water. It seemed possible that a careful examination of these latter might reveal some permeable spot in their cuticularized walls. Each gland consists of four cells arranged to form a broad truncate cone with both base and apex convex. The single basal cell is the largest, and bulges into the tissue of a leaf.

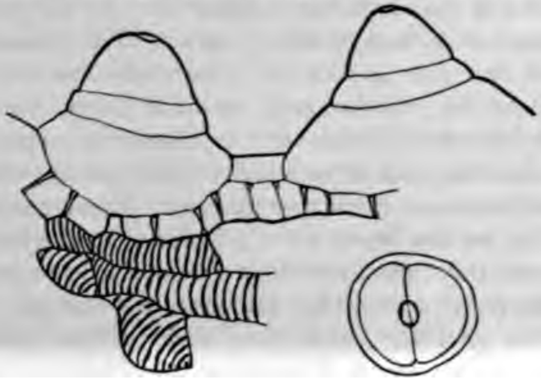

Fig 1. *Fig 2.*

Woodcut 7.

Above it succeeds a single discoid stalk-cell. The two remaining cells—the cap-cells—lie on the stalk-cell like two quadrants, roughly forming a hemisphere. The exposed walls of the gland are everywhere cuticularized, excepting at a circular spot in the centre of the apex at the junction of the two cap-cells. The cuticle is here interrupted by a large pore spanned by cellulose; but inasmuch as the cuticle is usually slightly raised round the margins of the pore, the latter in lateral optical section looks somewhat like a unilateral bordered pit. The pore is often blocked by a little plug of mucilage, which frequently protrudes outwardly in the form of a minute rounded knob. This mucilage in many of these glands contains solid calcium carbonate in small quantities. The external walls of the gland are marked with straight striations, which descend from the cap-cells to the basal cell as far as the general epidermis or to the point of contact of a contiguous gland. The cuticle is thickest on the wall of the stalk-cell. The transverse walls bounding the stalk-cell are thin, and consist of pure cellulose. The basal cell has conspicuous elongated pits on its lateral walls where they impinge on the basal cell of an adjoining gland. The fine vascular bundles have well-developed tracheides, and run close beneath the dome-shaped glands. When the bundle still possesses phloem (which therefore lies between the tracheides from the glands), a unique histological provision exists apparently for the purpose of directing the flow of water from the tracheides to the neighbouring glands. The cells of the nerve-parenchyma-sheath lying on the side away from the glands are closely set together without intercellular spaces; but tracing them up to the sides of the bundle towards the glands, they are continuous with series of thin-walled parenchymatous cells which end finally under the glands. Distinct intercellular spaces occur amongst these connecting cells. Where their walls connect them with their fellow-cells they consist of pure cellulose, but where the walls bound the intercellular spaces they are cuticularized. The result is that the nerve-parenchyma-cells in contact with the tracheides communicate with the basal cells of the glands by thin-walled cells whose intercellular spaces form a network completely lined with cuticle. This arrangement, I suggest, enables water to flow, even under pressure, from the tracheides to the distant glands without being forced into the intercellular spaces. Where the intercellular spaces reach the lower wall of a basal cell, the cuticularization spreads over that region of the basal wall. Hence the lower wall of a basal cell is cuticularized in patches which correspond to the intercellular spaces; but consists of pure cellulose where it touches the subjacent parenchyma. Even when the vascular bundles have lost their phloem, the tracheides being still separated from the glands by several layers of cells, the same peculiar system of cuticularized canals is often visible. When, however, the bundles dwindle to a line of tracheides separated from the dome-shaped glands by only one layer of parenchyma, I sometimes failed to detect cuticularization of the walls bounding the intercellular spaces. Near the margin of the leaf the tracheides are dilated and ampullate, and end against small parenchymatous cells which connect them with the basal gland-cells. These parenchyma-cells are always separated by considerable intercellular spaces: so that these marginal glands are built on precisely the same physiological plan as those in *Lathraea*, though not in the same histological manner.

RHINANTHUS CRISTA-GALLI.

As the excessively rapid discharge of water from the leaves of *Pedicularis* rendered it impossible to localize the exact source of the excretion of water, it seemed advisable to select a plant whose leaves possessed fewer dome-shaped glands. On this account I found *Rhinanthus Crista-galli* a suitable subject for investigation.

Each leaf is thick with indented margins which are slightly recurved towards the lower face. The nerves correspond with depressions on the upper face. The secondary nerves run from the midrib to the sinuses of the marginal indentations. The dome-shaped glands are very much less numerous than in the two preceding plants; they are limited to the lower face of the leaf, and only lie over the finest nerves, especially near the margin. The shortly-stalked capitate glands, distributed in the same manner, are very much more numerous, but they also occur in considerably smaller numbers in the furrows denoting the nerves, on the upper face of the leaf. Acuminate hairs are present on both faces of the leaf and on the margins. The stomata are numerous on the lower face, and a few occur on the upper face of the leaf.

Excretion of water by the Leaves. Plants rooted in grasssods, and cut shoots into which water was forced under pressure, when kept in a saturated atmosphere, both gave the same results as regards the excretion of water. Water was slowly poured out by the young leaves. The observations on the source of the excreted water were at first quite unexpected. Rarely could any water be seen on the lower faces of the leaves (where the dome-shaped glands occur); whereas it was constantly visible in the sunken channels marking the upper faces of the secondary nerves, and extended as a thin film to the marginal sinuses. At first it appeared that this water could not have been excreted by the dome-shaped glands: nor did it seem more likely that it could have been poured out by the capitate hairs or stomata, both of which are more numerous on the lower than on the upper face of the leaf. A few simple experiments cleared up the mystery. A shoot was held in an erect position with the leaves extended in the normal position, and a drop of water was placed on the lower face of an unmoistened leaf near the margin. The drop spread to the nearest marginal sinus, and extended itself in the form of a thin film along the sunken channel marking the upper face of the secondary nerve which supplied that sinus. A drop of water placed on the lower face of a leaf near the midrib (where no glands occur) did not move: it remained a drop. Finally, a drop of water placed on the upper face of a normally disposed leaf flowed along the sunken channels of the secondary nerves towards the channel of the midrib, but did not appreciably spread towards the margin. These experiments show, therefore, that in a normally excreting leaf, the water present in the peripheral portions of the nerve-channels in the upper face must have been excreted by the upper or lower face near the margin. Inasmuch as the histological details of the upper face of the leaf are essentially the same throughout, and there is no close connexion between the upper epidermis and the tracheides, nor any peculiarity in the epidermis near the margin, there is every reason to believe that the water is not excreted by the upper face. On the contrary, the portions of the lower face near the margins differ markedly from the parts near the midrib in their possession of dome-like glands, more numerous capitate hairs, and in the close histological connexion of these with the fine ends of the bundles.

Structure of the dome-shaped glands. These glands are built upon the same plan as those of *Lathraea.* Four cells with parallel lateral walls form a cap; a large biconvex cell lies below; and beneath it are four cells which, seen from above, look like the four quadrants of a disk, but have considerable round or elliptical intercellular space at the middle of each radius. The exposed walls of the gland are cuticularized everywhere save at the centre of the tip of the gland, where a pore like that of *Lathraea* occurs. The rim of the pore has a slightly thickened ring of cuticle, which often may be seen to project slightly outwards. A mucilaginous plug protrudes outwards in the form of a small hemisphere. In neither this plant nor in *Pedicularis* could I satisfy myself whether the plug is or is not perforated by an axial canal; but in *Rhinanthus* the plug, looked at from above, often seemed to have the appearance of being traversed by a vertical fine canaliculus. The tracheides and vessels underlying these glands are less developed and narrower than in *Lathraea* and *Pedicularis*, only near the leaf margin are the tracheides dilated. There is no cuticularization of the walls of the parenchymatous cells connecting the tracheides and the lowest series of gland-cells. The walls of the capitate hairs are externally cuticularized, there is no perforation of the cuticle.

ODONTITES RUBRA.

Rooted plants and cut shoots into which water was forced by mercury-pressure gave a very feeble excretion of water, even when kept in an atmosphere saturated with aqueous vapour. With the naked eye the drops could only be seen on the lower face of the leaf near the tip. My experiments with this plant were interrupted, consequently I can merely point out that the feeble excretion of water goes side by side with the numerical weakness in dome-shaped glands.

CONCLUSIONS.

So far I have shown that water is excreted in a liquid form by the leaves of *Lathraea*, *Pedicularis*, *Rhinanthus*, and *Odontites*, all of which possess dome-shaped glands on their lower faces. The following facts tend to prove that the water is poured out by the dome-shaped glands:—

1. The amount of water poured out is large when these glands are numerous (*Lathraea*, *Pedicularis*), and small when they are few in number (*Rhinanthus*, *Odontites*).

2. There are indications that only those portions of the leaf-surface which bear these glands excrete water (*Lathraea*, *Rhinanthus*).

3. The cuticle of these glands, in the three cases investigated, is perforated by a pore [*Lathraea* (1), *Pedicularis*, *Rhinanthus*] which renders that region of the wall permeable to water. I therefore regard this pore as a water-way, not as a channel through which mucilage may be excreted, as is generally supposed.

4. There is a close connexion between the tracheides in the fine bundles and these glands. I can confirm the accounts given by Scherffel (1), who alone of previous observers has correctly described the structure of these glands in *Lathraea* and their relations to the tracheides. As Scherffel points out, it is not true that a bundle of tracheides terminates under each gland. I find a network of tracheides very close beneath the epidermis lining the pocket. At certain points, especially at nodes of the network, certain broad ampullate tracheides bulge out towards the epidermis; but these lateral bulgings are less numerous than the dome-like glands, and, moreover, capitate hairs stand over them as well as dome-like glands. There is no trace of the cuticularization of the walls of subjacent parenchyma-cells where they bound intercellular spaces. In the plane scales on the floral axis of *Lathraea*, the dome-

like glands are only found just outside the ends of the fine nerves.

The excretion of water does not take place through stomata, for they are absent from the subterranean scales in *Lathraea*: nor through special water-stomata, which are absent in all these plants (excepting that the teeth-stomata of the flat scales of *Lathraea* may be water-pores). There is every reason to believe that the capitate glands are not appreciably responsible for the excretion of water, for they are most numerous where excretion is very slow (*Rhinanthus*), and show no intimate and necessary connexion with the ends of the vascular bundles. We are therefore entitled to assume that the *subterranean scale-leaves of Lathraea are the water-excreting organs of the subterranean plants*, and that this excretion of water is vigorous, inasmuch as the dome-shaped glands are numerous. This view is confirmed by a fact recorded by Mr. Gilburt (2) in his valuable little paper. Mr. Gilburt imagined that the dome-shaped glands excreted an acid sap which should serve to dissolve organic substances in the soil in order that the solution might be reabsorbed as food. This excretion of acid sap, he says, is abundant, for 'in the bank from which I have taken my material, and which is composed of a light, friable soil, the soil immediately surrounding the *Lathrea* (*sic*) was saturated with moisture, while all beside could be crumbled apart with the fingers.' A number of observers record that liquid as well as air is to be found in the pockets.

This view of the water-excreting function of the subterranean leaves of *Lathraea* is confirmed by reference to other allied types with subterranean scales. On these latter, as well as on the aerial leaves of the same plants, dome-shaped glands are found. I take the following anatomical facts mainly from Hovelacque's comprehensive work (3). *Bartsia alpina* is a root-parasite, usually living in humus-laden wet earth. Its aerial leaves have dome-shaped glands over the fine nerves near the margin of the lower face. The subterranean scales possess numerous dome-shaped glands and capitate hairs on the more peripheral portions of their lower faces. *Pedicularis sylvatica* grows in moist meadows. Its lowest, quite subterranean, leaves have backwardly recurved margins, and only under the reflected portions on the lower face are the numerous dome-shaped glands which are mingled with capitate hairs. Under these dome-shaped glands the fine nerves form an almost subepidermal network of tracheides. The foliage-leaves have recurved margins, and possess numerous dome-shaped glands on their lower faces, along the courses of the fine nerves: but the glands are not confined to the reflected portion. *Tozzia alpina* grows in wet alpine spots. The plane foliage-leaves possess on their lower faces, along the courses of the fine nerves, numerous dome-shaped glands and capitate hairs, which are especially abundant near the marginal sinuses. The wholly subterranean scales have their lateral margins very strongly reflected backwards, so that only small parts of their lower faces are visible. Numerous dome-shaped glands and capitate hairs clothe the inner (lower) surface of the reflected parts, and many dilated tracheides end directly under the epidermis of this region. The scales higher up have their lateral margins strongly reflected only on the more distal parts, and their basal portions are like the foliage-leaves as regards the disposition of the hairs. Thus these three Scrophulariaceous plants (the only ones on which I can find observations recorded with reference to subterranean scales and foliage-leaves), growing in moist or wet soils, have a specially well-developed system of dome-shaped glands. So far as I can judge from Hovelacque's descriptions and figures, it seems that there is a relative increase in the development of these glands in the subterranean scales compared with the aerial leaves: this is very clearly marked in *Tozzia* and in *Lathraea*. This recalls the increased development of the physiologically equivalent water-excreting mechanism to which I called attention in the subterranean parts of saprophytes (8). It further leads us to anticipate that ordinary green geophilous plants will possess some similar mechanism.

In all these cases, the considerable development of the water-excreting apparatus in subterranean parts is to be correlated with the fact that the nature of the environment of these parts is such as to depress or entirely arrest transpiration underground.

It has been frequently suggested that both the dome-shaped glands and the capitate hairs absorb nutrient liquids. Mr. Gilburt made a few experiments, and obtained evidence of the absorption of salts when supplied to these structures, and he therefore concluded that they naturally absorb solutions found in the soil. But a glance at Mr. Gilburt's statistics shows that the absorption of the salts was exceedingly slow, and in some instances could not be detected at all. I therefore regard his statistics as pointing out that the absorbent activity of the glands and hairs is so inconsiderable that we cannot suppose it to be of any fundamental importance to the plant. My own limited experiments confirm this view. By the use of very dilute solutions of gentian violet, by plasmolysis with common salt solution, or by staining with iodine (potassic-iodide solution) placed on an intact leaf of *Lathraea*, it was easy to show that the epidermis lining the pocket is more permeable to solutions than is the exposed epidermis. But only occasionally did I get any signs of a more rapid absorption of liquids by the dome-shaped glands than by the adjacent epidermal cells.

Object of the pocket-like form of the leaf. Cohn (4) suggested that these leaves might entrap and digest small organisms living in the soil, but he dismissed the view, as did Krause (5), Scherffel (1), and Heinricher (6): their evidence, together with the fact of the slow rate of absorption of liquids by the glands and hairs, is sufficient to show that the concavity of the leaves has nothing to do with the formation of a trap. I believe that the form of the leaf is adapted to protect the water-excreting glands (and possibly the capitate hairs), and is associated with the subterranean mode of life. The glands and hairs on the subterranean leaves have to contend, not only with climatic changes of one vegetative season, but have to endure the hardships of a winter, the dangers of saturation of the soil, and the percolative movements of the water in the soil. In confirmation of this view the following facts appear in reference to *Lathraea* and the parasitic Scrophulariaceae previously described in this paper:—(1) the dome-shaped glands are frequently confined to the recurved part of the leaf; (2) the glands are always very numerous in the strongly recurved types of leaves; (3) considering any particular plant, the relative number of glands on the leaf-surface is proportionate to the curvature of the leaf; (4) the curvature is strongest in the subterranean leaves. Even when the curvature is not more marked in the subterranean scales, chambers lined by glands are formed by the appropriate arrangement of the scales, at any rate in some plants (*Bartsia*).

Additional functions of the scale-leaves of Lathraea. I can add nothing to the information given by previous observers with reference to the capitate hairs. The leaves excrete a certain amount of chalk. Their fleshy mesophyll contains a great quantity of large grains of starch.

Conclusion. The pocket-like leaves of *Lathraea* are excretory organs and carbohydrate-reservoirs. Their concavity of form is assumed for the purpose of protecting the subterranean excretory mechanism; their succulence is associated with the necessity for providing house-room for the rich stores of reserve starch.

This research was conducted in the Botanical Laboratory of the University of Oxford.

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POSTSCRIPT.

This investigation was concluded in June, 1896, and the present paper was written, exactly as it now stands, in the autumn of the same year, but its publication has been unavoidably delayed. In the meanwhile, G. Haberlandt[1] and Goebel[2] have almost simultaneously published papers on *Lathraea*. Both these investigators agree with me that the subterranean scales of *Lathraea* excrete water into their chambers, and that the specific function of some of the glands lining these cavities is to rid the plant of an excess of water. Haberlandt concludes that the capitate hairs alone are responsible for the excretion of water: whereas Goebel suggests that this function is performed especially by the dome-shaped glands (*Schilddrüsen*). My paper, I think, contains sufficient proof that it is the dome-shaped glands which are the definite water-excreting organs—the hydathodes.

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[1] G. Haberlandt, Zur Kenntniss der Hydathoden: Jahrb. f. wissensch. Bot., xxx, Heft 4, 1897.

[2] Goebel, Ueber die biologische Bedeutung der Blatthöhlen bei Tozzia und Lathraea: Flora, Bd. lxxxiii, Heft 3, 1897.

The Anatomical Characters presented by the Peduncle of Cycadaceae.

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With Plates XX and XXI.

INTRODUCTION.

THE peculiar structure of the vascular bundles in the leaves of Cycadaceae has been well known to botanists since its discovery by Mettenius in 1860[1]. The peculiarity depends on the position of the spiral tracheae, which are the first formed elements (protoxylem) of the wood. In ordinary collateral bundles, such as those in the stem of Coniferae, and in both stem and leaf of most Angiosperms, the spiral elements lie at the extreme inner edge of the wood, remote from the phloem. Thus the whole development of the xylem, starting from the spiral tracheae, and advancing towards the phloem, proceeds in centrifugal order. In the foliar bundles of Cycads, on the other hand, the spiral elements are placed in the interior of the strand of xylem, but nearer the outer

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[1] Mettenius, Beiträge zur Anatomie der Cycadeen; Abhandl. d. K. Säch. Gesellsch. d. Wiss., Bd. vii, p. 567, 1860.

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than the inner surface. Here, then, the development goes on in two directions; a part of the wood is centrifugal, i.e. developed outwards towards the phloem, but the greater portion is centripetal, i.e. developed inwards, in the direction away from the phloem. This type of bundle may conveniently be called *mesarch*, a term suggested by Count Solms-Laubach[1], implying that the starting-point of the wood, the protoxylem, lies in the interior of the xylem-strand. The structure was thoroughly investigated by Mettenius, to whose observations little has been added by subsequent authors[2].

The relative development of the two parts of the wood varies considerably; in the finer veins of the foliage-leaves the centrifugal part may die out altogether, while in many sporophylls it greatly exceeds in bulk the centripetal portion, which may even be entirely absent.

Among recent plants typical mesarch bundles have, until the present time, only been found in the leaves of Cycadaceae[3]. In the stem of these plants only normal collateral bundles have so far been found. Mettenius has shown in detail how the transition from the one type of structure to the other takes place gradually in the base of the leaf[4].

Among fossil plants mesarch structure was much more widely spread. Not only did it occur in families belonging to the Cycadean stock, as in Bennettiteae and Medulloseae, but it was general in the leaves of the Cordaiteae, a fourth order of Gymnosperms, only known from the Primary Rocks[5].

The Cordaiteae, like the recent Cycadaceae, had normal bundles in their stems, but it is a remarkable fact that in certain other Palaeozoic plants the vascular bundles of the stem had a typically mesarch structure. This was notably the case in *Lyginodendron Oldhamium*, where the very perfect preservation enabled us to examine the anatomy in great detail, and to prove that the bundles of the stem are precisely similar in organization to those of Cycadean leaves[1]. The same was the case in *Poroxylon*, as shown by the investigations of MM. Bertrand and Renault[2].

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[1] Fossil Botany, English Edition, p. 257.

[2] See the well-known figures of this type of vascular bundle in De Bary's Comparative Anatomy of Phanerogams and Ferns, Figs. 158 and 159.

[3] Mr. W. C. Worsdell has quite recently shown that the bundles of the cotyledons and other leaves of *Ginkgo biloba*, and of *Cephalotaxus*, are mesarch, and finds reason to believe that in Conifers generally the 'transfusion-tissue' of the leaf is, in part at least, homologous with centripetal xylem. See his preliminary paper 'On the Origin of Transfusion-tissue,' Journal of the Linnean Society, 1897.

[4] l. c. p. 577.

[5] Renault, Tiges de la Flore Carbonifère, p. 295, 1879, Plate XVI.

Heterangium agrees with *Lyginodendron* so far as the leaf-trace-bundles are concerned[3]; and the *Calamopitys Saturni* of Unger, according to the recent investigations of Count Solms-Laubach, conforms to the same rule[4].

The frequency of mesarch structure in the stems of Palaeozoic plants showing unmistakable affinities with Cycadaceae, renders it highly probable that this character formerly extended to the stem, as well as the leaf, of the Cycadaceae themselves. This consideration suggested a renewed investigation of the anatomy of recent Cycads, in order to ascertain whether some vestiges of mesarch structure might not still survive in the bundles of the stem. The inquiry thus undertaken at once led to the observation that in the peduncle of both the male and female cone of *Stangeria paradoxa*, T. Moore, centripetal xylem is present, so that the structure of the vascular bundles in these axial organs is of the mesarch type[5]. This discovery has since been extended to the peduncles of certain other Cycads, namely *Bowenia spectabilis*, Hook., *Zamia Loddigesii*, Miq., and *Ceratozamia mexicana*[6], Brongn.

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[1] Williamson and Scott, Further observations in the organization of the Fossil Plants of the Coal Measures, Part III, *Lyginodendron* and *Heterangium*: Phil. Trans., Vol. 186, B, 1895, p. 713, Plates XXI and XXII.

[2] Recherches sur les Poroxylons: Archives Bot. du Nord de la France, 3me Année, 1886, p. 382.

[3] Williamson and Scott, loc. cit. p. 749.

[4] Solms-Laubach, Pflanzenreste des Unterculm von Saalfeld: Abhandl. d. K. Preuss. Geol. Landesanstalt; neue Folge, Heft 23, p. 65, Plate IV, 1896. The *Calamopitys* of Unger, which evidently belonged to the Lyginodendreae, must not be confused with the Calamarian stem described by Williamson under the same name: Mem. Lit. and Phil. Soc. of Manchester, ser. 3, Vol. iv, 1869.

[5] See Williamson and Scott, loc. cit. p. 768.

[6] In these three cases the centripetal xylem was first detected by my assistant,

It is in the male peduncle of *Stangeria* that the centripetal wood reaches its greatest development, and this case will therefore be first described.

STANGERIA PARADOXA, *T. Moore.*

General Structure. The principal vascular bundles of the peduncle, varying from 12 to 18 in number, are ranged in a single ring, which is fairly regular in the upper and middle part of the organ, but becomes much distorted towards its base, where the orientation of the bundles also becomes irregular (cf. Figs. 1 and 2). Lower down still, where the peduncle is about to pass over into the main stem, of which it forms the direct prolongation[1], the vascular ring once more becomes perfectly regular, but with a much smaller diameter, and a reduced number of bundles. Some peculiarities in the course of the vascular strands will be further considered below.

The structure of a normal main bundle is as follows. The centrifugal wood in the mature peduncle reaches a radial thickness of about 12 elements; the scalariform tracheides are closely packed, though the mass is broken up here and there by bands of parenchyma, which do not always form continuous medullary rays. On the inner side of each bundle there are two or more groups of spiral or annular tracheides, constituting the protoxylem. These elements soon become crushed by the growth of the turgescent parenchyma around them (see Figs. 3 and 4, *px*). The elements of the centrifugal wood do not show such regular seriation as in some other Cycads, though their number is certainly added to, for a long time, by the activity of the cambium.

The phloem is extremely well developed, and usually exceeds the xylem in bulk. Many of the individual sieve-tubes are larger than the tracheides, especially in radial

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Mr. W. C. Worsdell, who prepared numerous sections of peduncles, at my request.

[1] Solms-Laubach, Die Sprossfolge der Stangeria und der übrigen Cycadeen: Bot. Zeit. 1890.

diameter (see Figs. 3, 4, 5, and 7). The compound sieve-plates, chiefly on their radial walls, come out very clearly under appropriate treatment.

The small albuminous cells, which in Gymnosperms represent functionally the companion-cells of the higher plants[1], are very conspicuous, often forming regular tangential bands. On the outer side of the bundle is a band of crushed tissue, consisting of the obliterated elements of the first formed bast, or protophloem. Beyond this there is a small-celled parenchymatous tissue, which is no doubt to be regarded as pericycle. This tissue retains the power of cell-division for a long time, and occasionally gives rise to anomalous formations in connexion with the older bundles.

On the inner side of each bundle is a considerable mass of small, rather thin-walled cells, with conspicuous nuclei, forming a group easily distinguishable from the large-celled tissue of the pith (Figs. 4 and 5, *c*). This group is similar in structure to the pericycle, with which it is in fact connected, round the sides of the bundle. It no doubt comes under the category of M. Flot's internal pericycle, or 'zone périmédullaire,' the present case being one of those in which this zone is only differentiated in connexion with each separate bundle[2]. It is in this tissue that the centripetal xylem is developed.

Centripetal wood. The longitudinal distribution of the centripetal xylem is as follows. It is absent from the actual base of the peduncle, but begins to appear a little higher up, in the region where the irregularity of the vascular ring is most marked (see Fig. 2 *x*[1]). Above this point it rapidly increases in amount, and then maintains about the same degree of development throughout the greater part of the peduncle, in the region where the arrangement and orientation of the bundles is normal; in the axis of the cone itself the centripetal xylem again diminishes in amount, disappearing altogether towards the apex. Fig. 1 gives a fair idea of the extent of its development in the middle part of the peduncle. In this region almost every bundle shown in any transverse section possesses it, and often in considerable amount; at least two dozen elements of centripetal xylem often occur in connexion with a single bundle (see Figs. 3 and 4, x^1). Probably every vascular bundle in the peduncle has centripetal xylem in some part of its course.

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[1] Strasburger, Hist[illegible] Beiträge, III, pp. 154, 157.

[2] See [illegible] zone périmédullaire [illegible] Ann. des Sc. Nat. [illegible]

The nature of these internal lignified elements is shown in longitudinal sections, which prove that they are, as a rule, scalariform tracheides, like those which form the greater part of the centrifugal wood (see Figs. 5 and 7). The centripetal tracheides are, on the whole, shorter than the centrifugal.

The position of the centripetal xylem is such that its elements are always placed within the circummedullary zone; in some cases they lie at its inner margin, separated by a considerable interval from the spiral tracheides (Fig. 5); in other cases they are situated further to the exterior, and are sometimes almost in contact with the protoxylem (see Figs. 3 and 7).

The extent of the centripetal xylem and the arrangement of its elements in the peduncle of *Stangeria* is precisely comparable to that found in certain foliar organs of Cycadaceae, as, for example, in the stalk of the carpel of *Cycas revoluta* and other species of *Cycas* and of *Encephalartos*. The comparison of the structures, as shown, for example, in Fig. 3, from the peduncle of *Stangeria*, and Fig. 6, from the carpel of *Cycas*, can leave no doubt that the internal tracheides in the former are really homologous with the well-known centripetal xylem of the foliar bundles of Cycadaceae.

The centripetal elements which approach the protoxylem are, on the whole, smaller than those remote from it, just as in the case of the centrifugal wood (Fig. 4). The protoxylem-elements of the peduncle are usually in contact with the centrifugal part of the wood, whereas in the foliar organs of Cycads the reverse is more commonly the case. There are, however, so many exceptions to the latter rule, especially in the vascular bundles of the sporophylls, that no importance can be attached to this difference[1].

Centripetal xylem was found in all the male peduncles examined—five in number—which approached maturity. It is, however, developed late, as was proved by the comparison of a series of transverse sections, extending from base to top of a young peduncle, with corresponding series from more advanced specimens. The centrifugal wood of the young peduncle had attained a thickness of three or four elements, but in no part was any centripetal wood as yet differentiated. In another peduncle, with centrifugal wood five or six elements thick, the centripetal elements were already fairly numerous; they only attain their full number, however, in nearly mature peduncles, when the centrifugal xylem has from seven to twelve tracheides in each radial series. This late development of the centripetal wood accounts for the shortness of its tracheides, for at the time when they develop the tissue is too mature to allow of any great degree of sliding growth. For the same reason, spiral elements are rare, on the centripetal side, for as growth in length is usually over when these tracheides are differentiated, no provision for extensibility is needed. In one case, however, a very characteristic spiral tracheide, with a fairly close coil, lay between the disorganized protoxylem and the scalariform elements of the centripetal wood (Fig. 7 i). Here then there was a gradual transition from the primitive to the definitive tracheides, in both directions, but as a rule the centripetal xylem develops too late for such a transition to be traced.

The more internal tracheides of the centripetal wood are sometimes met with while still in course of development, as is best shown in radial sections.

This belated appearance of the centripetal wood in the peduncle, is the only point of any importance in which it differs from the corresponding tissue in the leaf. This, however, proves nothing against the homology between the two. In the peduncle of Cycads the centripetal part of the wood is a vestigial structure, and it is well known that vestigial organs, when recognizable in the adult, are usually late in completing their development.

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[1] In the petiole of [illegible] [illegible] [illegible], [illegible] the petiole alone [illegible] the protoxylem [illegible] both parts of the wood equally; the same is the case in the petiole of [illegible] [illegible] *Macrozamia* [illegible], [illegible] as well as in the petiole of *Stangeria*. In the [illegible] the spiral elements [illegible] was between the two [illegible] (Mettenius, [illegible] p. [illegible]).

Even in the foliar organs, the relative periods of development of the two parts of the wood is extremely variable, as is indicated by their variable relations to the protoxylem-elements. In the sporophylls of many Cycads the centripetal portion of the wood is excessively reduced and may even be altogether absent. In such cases the spiral elements are always in immediate connexion with the centrifugal wood (see Figs. 12 and 13). In the carpel of *Ceratozamia* the centripetal wood is developed late compared with the centrifugal portion, and no doubt this is often the case, where the former tissue is reduced.

The peduncle and axis of the female cone of *Stangeria*, like those of the male, show centripetal xylem in connexion with some of the bundles. In the specimens available, however, it was much less developed than in the male cone. Here also the elements in question are shown, by longitudinal sections, to be scalariform tracheides.

As a rule, the centripetal wood, whether in the male or female peduncle, is limited to the principal bundles of the stele. No clear case of the presence of this tissue in a leaf-trace passing out to a functional sporophyll has been observed, nor is this surprising, considering the excessively slight development of centripetal xylem in the bundles of the sporophylls themselves. In some cases certain small bundles, passing through the cortex, were found to have a few centripetal xylem-elements; but such bundles appear to be either purely cauline, or to be destined for the bracts or sterile sporophylls which occur at the base of the actual cone. In these the centripetal xylem is somewhat better developed than in the fertile sporophylls.

Vascular System. Before leaving *Stangeria* certain other peculiarities of the vascular system in the peduncle must be considered.

Throughout the whole length of the organ the main bundles fuse and separate again repeatedly, forming collectively a hollow network, with long meshes, as described by Count Solms-Laubach[1]. The number of the strands, as seen in transverse section, thus remains approximately constant, except at the base of the peduncle and the apex of the cone, where it diminishes greatly. In the lower part of the peduncle, where the ring of bundles becomes flattened and contracted, every kind of irregularity in their arrangement and orientation may be observed (see Fig. 2)[2].

Often the xylem forms a horse-shoe, opening inwards, with the phloem in its convex side (see Fig. 2, strand 4). In other cases two bundles lie close together with their phloem-masses in contact (Fig. 2, strands 1, 1). Comparison of successive transverse sections shows that the former arrangement is due to the fusion of two bundles from above downwards, while the latter case indicates that two bundles are about to fuse from below upwards. Occasionally the horse-shoe form becomes exaggerated, so that the xylem appears as a ring, nearly or quite surrounded by phloem. In fact a concentric structure may be acquired for the moment, but this peculiarity is quite local, and dependent simply on the fusion of the bundles; no morphological importance can, I think, be attached to it.

At a still lower level in the peduncle all the irregularities again disappear; the bundles, reduced in number, once more form a perfectly regular ring of small size, with typically collateral structure and normal orientation. The abnormalities of the transitional region are probably due to mechanical causes, the tissues developing under pressure from the leaf-bases between which this part of the peduncle is wedged in

On the other hand the small cortical strands which occur in the peduncle, especially in its lower part, present some features of interest. They resemble the sporophyll-traces in the fact that each strand, after branching off from the main ring, often divides into two branches, which reunite higher up[1]. In many cases these cortical bundles are altogether cauline, not passing out into any kind of appendage. Their course has been carefully followed in series of successive sections, from several peduncles, and it has been proved that they sometimes end blindly in the cortical parenchyma, and sometimes turn inwards again, to reunite with the main vascular bundles of the stele. Probably all the cortical strands, which occur in the lower part of the peduncle, are of this cauline nature. They possibly represent the vestiges of a former leaf-trace system, which, now that the greater part of the peduncle is naked, no longer has any leaves to supply.

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[1] [illegible] Stangeria, p. [illegible]

[2] [illegible]

Concentric Bundles. In some cases the cortical strands show a typically concentric structure, which they retain for a considerable distance. The longitudinal course of these concentric bundles has been followed in several instances, and one of them was traced through the greater part of the length of a peduncle. Low down, in the region of irregular orientation, a horse-shoe bundle was seen leaving the main ring. As it ascended, the curvature increased, until the structure became truly concentric, with xylem inside and phloem outside. The bundle remained concentric, but diminished in size, until, in the upper, regular, part of the peduncle, it turned in again, coming into contact with one of the main bundles, where it once more increased in bulk, but retained perfectly concentric structure.

The transverse sections of two such bundles (from different specimens) are shown in Figs. 8 and 9, from which their strictly concentric structure, with central protoxylem, is evident. The parenchyma in the centre of the strand is no doubt homologous with the circummedullary tissue accompanying the normal bundles.

These concentric cortical strands are of very inconstant occurrence, some peduncles not showing them at all. So far as I have observed they never split into two, as is so often the case with the collateral bundles in the cortex. Possibly their concentricity is due to this fact, the concentric strand thus representing a double bundle, just as occurs sometimes in the main ring. As we have already seen, a bundle which is collateral below may become concentric higher up; sometimes the change is in the reverse direction.

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[1] Cf. Solms-Laubach, l.c. p. 213.

In any case these cortical strands are of considerable interest, for they constitute, so far as I am aware, the only instance hitherto described of Cycadean bundles with a primarily concentric structure, retained for a considerable distance. They have been observed in quite young peduncles, so there can be no doubt that the concentric structure exists while the tissues are still in their primary condition[1].

The secondary concentric strands, so well known in the cortex of *Cycas*, clearly belong to a different category.

The evidence for the origin of Cycads from Ferns is now overwhelming, Professor Ikeno's discovery of the multiciliate spermatozoids of *Cycas* completing the proof in the most striking manner[2]. Hence one is tempted to look on concentric vascular bundles in a Cycad as a relic of ancestral structure. Such an inference must be received with caution, but I give the facts for what they are worth.

Other Anomalies. One or two occasional anomalies remain to be noticed. In rare cases a cambium arises in connexion with a group of the centripetal xylem, and a little secondary phloem is then found on the side toward the pith. I attach little or no importance to such isolated abnormalities of development, but it may be worth while to point out that similar anomalous formations, on a much larger scale, are frequent in the fossil *Lyginodendron Oldhamium*, and are even more marked in the form which Mr. Seward has named *Lyginodendron robustum*[1].

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[1] Mr. W[illegible] [illegible] Cycads, [illegible]

[2] [illegible] 1897; Annals of Botany, June 1897. [illegible] another [illegible] than the same [illegible]

As already mentioned the cells of the pericycle outside the bundle often become merismatic, dividing tangentially so as to produce a certain amount of new tissue, which usually remains parenchymatous, but which, in very rare cases, may give rise to a rudimentary vascular strand.

The chief point, however, which has been brought out by the anatomical investigation of the peduncle of *Stangeria* is the presence of centripetal xylem which, in the case of the male cone, may attain a development equal to that in such foliar organs as the carpels of *Cycas*, and greatly exceeding that in most other Cycadean sporophylls. The general bearings of this result will be discussed after some other cases have been considered.

BOWENIA SPECTABILIS, *Hook.*

In the peduncles of both male and female cones of *Bowenia*, centripetal xylem occurs, but only to a very small extent. There is a great difference in the size of the peduncles, that of the female cone being much the thicker, with larger and more numerous vascular bundles than those in the male peduncle: otherwise the structure is identical.

There is nothing peculiar in the centrifugal xylem or the phloem; here, as in *Stangeria*, the phloem is decidedly the more developed of the two tissues. Centripetal wood was only observed in a few of the bundles, and not more than four such elements were found in connexion with any one strand. They are separated from the protoxylem by from one to three layers of cells. Longitudinal sections show that here, also, the elements of the centripetal wood are scalariform tracheides. The tissue is present in such small quantity, that, but for the analogy of *Stangeria*, it might easily be overlooked. There is no doubt, however, as to its homologies, but in *Bowenia* the centripetal xylem of the peduncle is evidently in the last stage of reduction.

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[1] Williamson and Scott, loc. cit. Part III, p. 722, Plate XXIII, Fig. 8: Seward, A Contribution to our knowledge of *Lyginodendron*, Annals of Botany, March, 1897.

ZAMIA LODDIGESII, *Miq.*

The peduncle of a female cone of this species was examined, and, next to *Stangeria*, afforded the best example observed of centripetal wood. Towards the base of the peduncle most of the vascular bundles possess this tissue, and in some it is very well developed (see Fig. 10, x^1). The centrifugal wood and phloem show a very regular radial seriation; there are usually several groups of spiral tracheides to each bundle. The elements of the centripetal xylem are, as a rule, found at a little distance from the protoxylem, towards the inner margin of the small-celled circummedullary zone. The elements of the centripetal wood, like those which form the bulk of its centrifugal portion, are scalariform tracheides (see Fig. 11). They have a rather wavy course, and are often quite short, with truncated ends, especially on the side remote from the protoxylem. Both these peculiarities are no doubt due to late development, taking place at a stage when the tissues are too mature to allow of the unrestricted growth in length of the newly differentiated tracheides.

Occasionally a little centripetal xylem occurs in connexion with the small leaf-trace bundles passing through the cortex.

In a male peduncle of the same species no centripetal wood could be detected.

CERATOZAMIA MEXICANA, *Brongn.*

In the peduncle of the male cone of this species fairly well-marked centripetal xylem sometimes occurs in connexion with the principal vascular bundles. It was found best developed in the axis of the cone itself, but it also occurs throughout the whole length of the peduncle down to its base. About half a dozen centripetal tracheides may be present in connexion with a single bundle, but their occurrence is very inconstant.

The course of the bundles was traced in peduncles which remained attached to a very old stem of this species, embedded among the leaf-traces. They had lost their cones some time before, but were probably of no great age, as they occurred near the apex, and their structure was still intact. The general vascular arrangement in the lower part of the peduncle is essentially the same as in *Stangeria*. Quite at the base there is a small ring (about 3 x 4 mm. in diameter) of perfectly normal bundles; a little higher up the ring enlarges, and becomes more elliptical, measuring about 10 x 5 mm. The bundles at the narrow ends of the ellipse are crowded and irregularly orientated—obviously as the result of development under pressure from the leaf-bases [1]. A more remarkable anomaly is the presence of inverted cortical bundles immediately outside those of the normal ring. Some of these bundles, where they pass further out into the cortex, show some approximation to a concentric structure.

Further up still, the ring of bundles again becomes regular, but as the cone is approached, new peculiarities make their appearance. The principal bundles are no longer equidistant from the centre but form an irregular double ring, with variable orientation of the individual strands. Small bundles enter the pith; when traced up into the axis of the cone they are found to pass out, usually fusing, as they do so, with the principal strands. The same arrangement was shown in a male cone of *C. latifolia*, Miq., but it does not appear to be constant throughout the genus. In *C. latifolia* also, slight traces of centripetal xylem were observed.

The peduncles of a number of other Cycadaceae of the genera *Cycas* (♂), *Macrozamia*, *Ceratozamia*, and *Zamia* were examined, without, so far, finding any other clear case of centripetal xylem. The instances, however, in which it has been observed, are so widely scattered throughout the Order, that other examples will undoubtedly be added on further investigation.

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[1] Cf. Solms-Laubach, l. c. p. 212.

CONCLUSION.

The most important result of these observations has been to establish the fact that the mesarch type of vascular bundle, hitherto believed to be limited to the leaf in recent Cycadaceae, also occurs in certain axial organs, namely in the peduncles of *Stangeria*, *Bowenia*, and some species of *Zamia* and *Ceratozamia*. Count Solms-Laubach, as the result of his work on the course of the vascular bundles of Cycads, came to the conclusion that in these plants the peduncle shows a primitive organization, as compared with the vegetative stem; he says: 'Sehr merkwurdig aber ist die Art und Weise, wie an einem und denselben Spross unter plotzlicher Verjungung des Pleromkorpers der complicirte vegetative Spurverlauf ganz unvermittelt in den einfachen der Bluthe uberspringt. Dass dieser letztere *eine Reliquie uralter Organisation*[1], dass er den gemeinsamen Vorfahren der Cycadeen und Bennettiteen allgemein eigen gewesen sein wird, dass der vegetative Spurverlauf, wie er jetzt bei ersterer Gruppe vorliegt, eine im Laufe der Zeit erworbene Eigenschaft darstellt, die den Gang der Entwicklung in der Richtung vom Einfachen zum Complicirten uns vor Augen fuhrt, scheint mir eine sehr nahe liegende Annahme zu sein.'

My observations on the structure of the vascular bundles in the peduncle completely confirm Count Solms' conclusions, drawn from their arrangement. In the presence of centripetal wood, so well marked in the peduncle of *Stangeria*, and evident, though less conspicuous, in other cases, we have the same character, in a reduced form, which is presented by the vegetative stem of the fossil plants *Lyginodendron* and *Poroxylon*, as well as by the *Calamopitys Saturni* of Unger.

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[1] The italics are my own.

It appears then that the Cycadean type of vascular bundles—one of the most ancient of anatomical characters—was not originally a mere peculiarity of the leaf, but rather represents the vestige of a primitive organization, which was once common to the stem also[1].

In recent Cycads the stem has all but lost this character; it survives, so far as we know, only in certain peduncles, organs which appear to have been less modified than the vegetative stem.

The loss of the centripetal wood was in all probability correlated with the increasing development and earlier appearance of secondary tissues. The earlier the stage at which the formation of wood by the cambium set in, the more would the primary centripetal xylem become superfluous. We can form an idea of the progress of this change by the comparison of some of the fossil forms. In *Heterangium* the secondary tissue-formation set in late, and was, as a rule, comparatively small in amount. In *H. Grievii* especially, the great bulk of the wood is primary, and nearly all the primary part is centripetal, occupying the whole interior of the stele. In *Lyginodendron Oldhamium* the central part of the wood has disappeared, and is replaced by a large pith, around which the bundles are ranged. In each bundle the centripetal portion of the wood is well developed, but secondary growth sets in early, and the secondary wood soon far exceeds the primary in amount[2].

In *Lyginodendron robustum*, Seward, no primary centripetal wood has been certainly demonstrated as yet; if present, it must have borne a very small proportion to the secondary tissues. In *Cycadoxylon* the former tissue seems to have all but disappeared, much as in recent Cycads[3].

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[1] This view has already been stated in the joint paper on *Lyginodendron* and *Heterangium* by the late Dr. Williamson and myself. See p. 768, l. c.

[2] For *Heterangium* and *Lyginodendron* see Williamson and Scott, loc. cit., and the earlier papers by Williamson there cited; also Seward, Palaeobotany and Evolution, Science Progress, October, 1896.

[3] See Seward, A Contribution to our knowledge of *Lyginodendron*, Annals of Botany, March, 1897. The author constantly uses the term 'centripetal wood'

As regards the development of the centripetal xylem, *Poroxylon* is about on a level with *Lyginodendron Oldhamium;* but in the former this tissue dies out towards the lower end of each leaf-trace bundle, about seven or eight internodes below the point where it enters the stem[1]. *Poroxylon* seems to show distinct affinities with the Lyginodendreae on the one hand, and the Cordaiteae on the other.

Ultimately the stem retained centrifugal wood only, as in the Cordaiteae, and in recent Cycads, with the exceptions recorded in the present paper. We must not suppose that the whole of the centrifugal wood was secondary in origin. In *Lyginodendron* and *Heterangium*, the primary xylem has a well-marked centrifugal portion[2]. The same is clearly the case in many foliar bundles of recent Cycads, though sometimes the limit between primary and secondary tissue may be impossible to define.

The view maintained in the present paper as to the evolution of the vascular bundles in Gymnosperms has much in common with that put forward by MM. Bertrand and Renault in 1886[3]. These authors, however, assumed that the whole centrifugal part of the wood is secondary, an assumption which hangs together with their attempt to derive Cycads, *Poroxylon*, and *Lyginodendron* from Lycopods and not from Filicineae. This view is no longer tenable. Both recent Cycads and their fossil allies (Bennettiteae, Lyginodendreae, Medulloseae) abound in Fern-like characters, and have simply nothing in common with Lycopods. *Lyginodendron*, for

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for the secondary tissue, sometimes developed at the margin of the pith in *Lyginodendron* and *Cycadoxylon*. This terminology is of course quite legitimate, but it is important to avoid confusing this anomalous secondary formation with the centripetal xylem which forms part of the primary structure of the bundle. The latter is simply termed 'primary wood' by Mr. Seward. On Cycadoxyleae see Renault, Flore fossile d'Autun et d'Épinal, Part 2, 1896.

[1] Bertrand et Renault, Recherches sur les Poroxylons, Archives Bot. du Nord de la France, 1886-7; also Remarques sur les faisceaux foliaires des Cycadées, loc. cit. p. 238.

[2] Williamson and Scott, loc. cit., pp. 713 and 749.

[3] Remarques sur les faisceaux foliaires des Cycadées, loc. cit.

example, had in all respects the foliage of a Fern, and so had *Heterangium*[1]; in the latter the structure of the stem, apart from the secondary growth, is exactly that of a monostelic fern, while the supposed resemblance to *Lycopodium* is merely fanciful. Recent work has completely confirmed Williamson's original view as to the Fern-affinities of these fossil genera.

While the origin of the Cycadean stock—in the widest sense—from Filicineae is now well established, it is not possible in the present state of knowledge to determine more exactly the line of descent of our recent Cycadaceae. Though they retain many primitive characters, they form but one special group, of what was once an extensive and varied class, which we may call Cycadales. The best known of the abundant Cycadeoid remains which have come down to us from the Secondary Period, are those of Bennettiteae, a group very distinct from the living Cycadaceae. Much additional palaeontological evidence will be required before the geological history of the latter family can be revealed.

The Cycadales generally may even be polyphyletic, having perhaps sprung from the Filicineae at various points. The Lyginodendreae, Protopityeae, and Medulloseae all combine Filicinean with Cycadean characters, but they appear to join on to very different groups of Ferns. In the light of Count Solms-Laubach's recent remarkable observations on the Cladoxyleae of the Culm[2], it seems highly probable that the Medulloseae sprang from polystelic Ferns, each of the well-known rings of wood in the Medullosean stem corresponding to a distinct stele, with its own secondary zone. On the other hand, the Protopityeae[3], and the Lyginodendreae, especially the latter, are essentially monostelic, *Heterangium* directly connecting *Lyginodendron* with the type of stem-structure now represented by *Gleichenia*, &c.

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[1] It is extremely probable that *Sphenopteris Höninghausi*, Brongn., was the foliage of *Lyginodendron Oldhamium*, and *S. elegans*, Brongn., that of *Heterangium Grievii*. Cf. Kidston, On the Fructification and Internal Structure of Carboniferous Ferns; Trans. Geol. Soc. Glasgow, Vol. ix, Pt. 1.

[2] Pflanzenreste des Unterculm von Saalfeld, above cited.

[3] Solms-Laubach, Bot. Zeitung, 1894, Abth. I, p. 206.

Recent Cycads and, so far as I am aware, the Mesozoic Bennettiteae also, have evidently monostelic stems, and the observations recorded above tend to connect them with the Lyginodendreae rather than with the other intermediate groups. On this point, however, it is necessary to speak with great caution, for the distinction between polystely and monostely—so striking in the extreme forms—is one on which it is quite possible to lay too great stress[1]. We may, however, safely go as far as this: the discovery of mesarch bundles in certain axial organs of Cycadaceae, establishes a new link between that family and the Lyginodendreae, though by no means excluding the possibility of relationship in other directions.

The other point of interest recorded above, is the occasional occurrence of concentric vascular bundles in the cortex of the peduncle. This is a very inconstant phenomenon, and I hesitate to attach great importance to it, for we know that concentric structure sometimes appears in the foliar bundles of various Angiosperms, where there can be no question of phylogenetic significance[2].

The cortical bundles of the peduncle in *Stangeria* appear to represent a vestigial leaf-trace system, no longer connected with leaves. It is possible that a primitive structure has sometimes been retained by these now rudimentary strands. The concentric structure suggests a comparison with Medulloseae, but a better analogy can be found among the Lyginodendreae, for the leaf-traces in the cortex of *Calamopitys Saturni* appear to have been perfectly concentric[3]. In *Lyginodendron* the leaf-traces were collateral, only becoming concentric in the leaf itself.

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[1] On this point see, for example, Mr. Gwynne-Vaughan's paper on Polystely in the genus *Primula*, Annals of Botany, June, 1897.

[2] Cf. Gwynne-Vaughan, loc. cit.

[3] Solms-Laubach, Pflanzenreste des Unterculm, Plate IV, Fig. 11.

The main results attained may be briefly summed up thus:—

1. The peduncles of certain Cycads (*Stangeria paradoxa*, ♂ and ♀; *Bowenia spectabilis*, ♂ and ♀; *Zamia Loddigesii*, ♀; *Ceratozamia mexicana* and *latifolia*, ♂) have mesarch vascular bundles, comparable in structure to those of the leaves. This is, in my opinion, a primitive character, indicating affinity with the fossil Lyginodendreae and Poroxyleae.

2. Some of the primary cortical bundles of the peduncle of *Stangeria* show concentric structure. *Possibly* this also may be a relic of ancestral organization.

In conclusion I desire to express my thanks to Mr. W. C. Worsdell, to whose skilful aid in the investigation I am greatly indebted. The illustrations are also from his hand.

EXPLANATION OF FIGURES IN PLATES XX AND XXI.

Illustrating Dr. Scott's paper on the Peduncle of Cycadaceae.

The following abbreviations are used throughout the figures:—*px.* protoxylem; x^1. centripetal xylem; x^2. centrifugal xylem; *ph.* phloem; *cb.* cambium.

PLATE XX.

Fig. 1. *Stangeria paradoxa.* Diagrammatic transverse section of the peduncle of a male cone, taken about half way up, showing the arrangement of the vascular bundles, and the distribution of the centripetal wood, indicated by dots; *c. s.* cortical strands. The numerals indicate corresponding bundles in this section and the next. × about 12.

Fig. 2. *Stangeria paradoxa.* Similar section from the lower part of the same peduncle, at the level where the centripetal wood first appears. Note the irregular orientation of the bundles in this region. *c. s.* cortical strands, one of which is concentric. × about 12.

Fig. 3. *Stangeria paradoxa.* Transverse section of a vascular bundle from the upper part of a male peduncle. The centripetal xylem is well developed, and abuts on the compressed protoxylem. × 85.

Fig. 4. *Stangeria paradoxa.* Similar section from the middle part of another male peduncle. *c.* small-celled tissue in which the centripetal xylem is situated; *p.* pith. × about 85.

Fig. 5. *Stangeria paradoxa.* Radial section through a vascular bundle in a male peduncle. The centripetal xylem is here shown at the border of the pith, *p*, separated by narrow-celled tissue, *c*, from the spiral tracheides, *px*. × about 85.

Fig. 6. *Cycas revoluta.* Transverse section of a vascular bundle from the stalk of a carpel, for comparison with Fig. 3, from the peduncle of *Stangeria.* To the left a small inverted cortical bundle is shown. × 85.

PLATE XXI.

Fig. 7. *Stangeria paradoxa.* Radial section through a vascular bundle in a male peduncle. The centripetal xylem begins with a spiral tracheide. × 130.

Fig. 8. *Stangeria paradoxa.* Transverse section of a concentric cortical bundle from the middle part of a male peduncle. × 130.

Fig. 9. *Stangeria paradoxa.* Similar bundle, from the lower part of another peduncle. There is here a central parenchyma in which the protoxylem is embedded. Sieve-plates are indicated on the radial walls of some of the sieve-tubes. × 130.

Fig. 10. *Zamia Loddigesii.* Transverse section of a vascular bundle from the peduncle of a female cone, showing centripetal xylem. × 85.

Fig. 11. *Zamia Loddigesii.* Radial section of a similar bundle. × 85.

Fig. 12. *Stangeria paradoxa.* Transverse section of a vascular bundle from a carpel. The centripetal xylem is very slightly developed. × 110.

Fig. 13. *Stangeria paradoxa.* Similar bundle from a stamen. × 110.

Fig. 1.

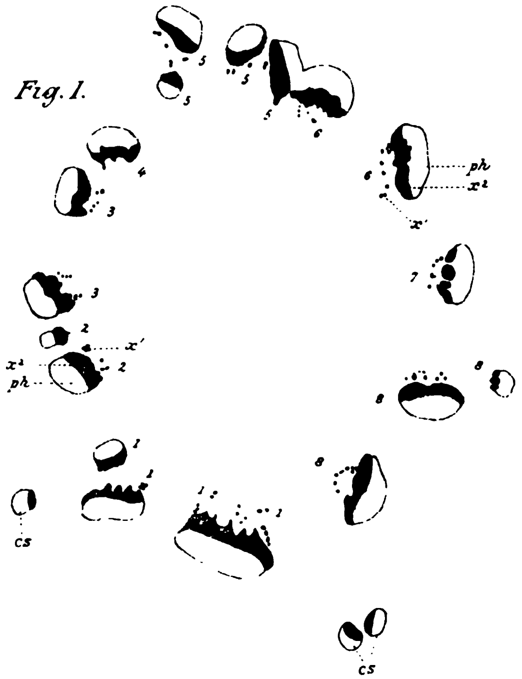


Fig. 2.

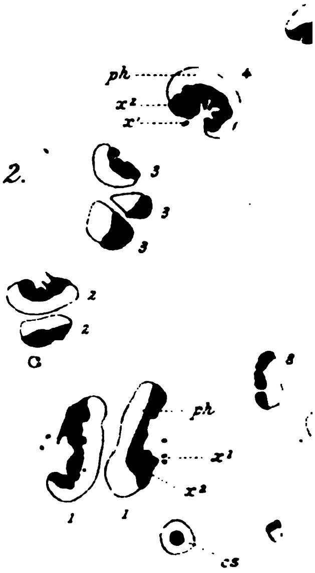


Fig. 4.

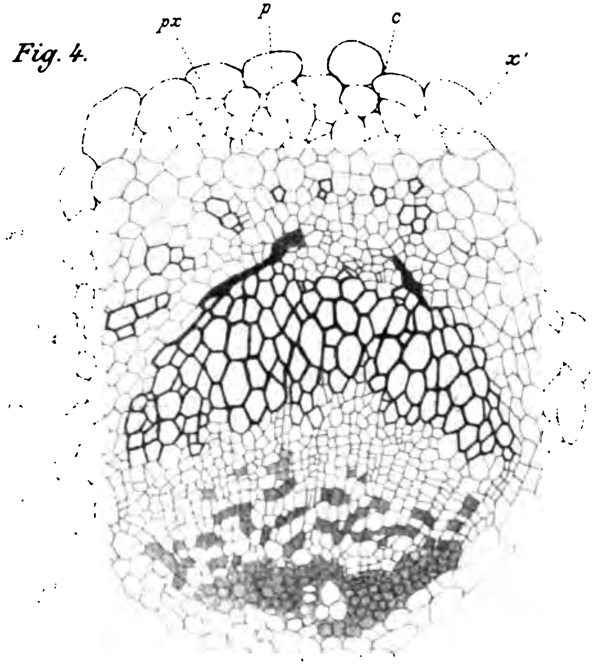
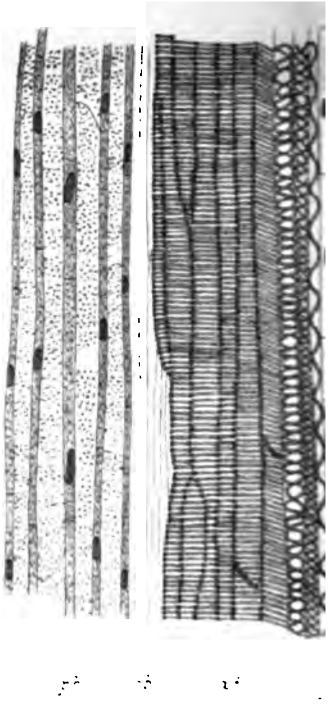


Fig. 5.



SCOTT — PEDUNCLE OF CYCADACEÆ.

Fig. 5

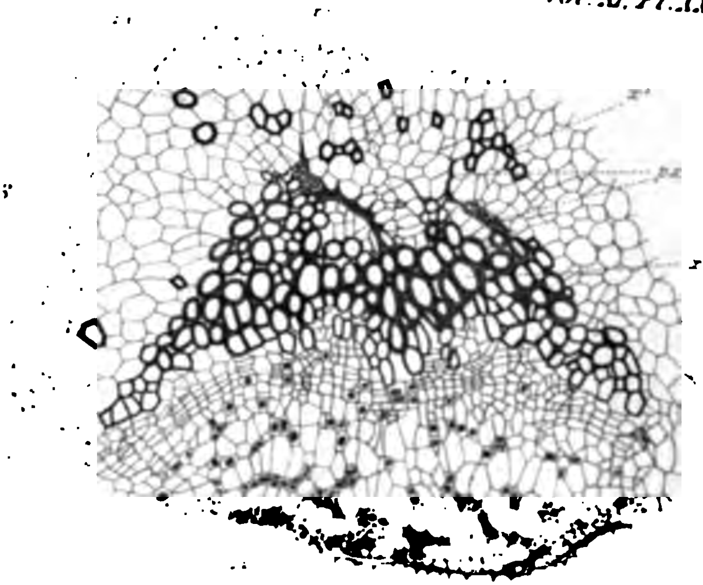
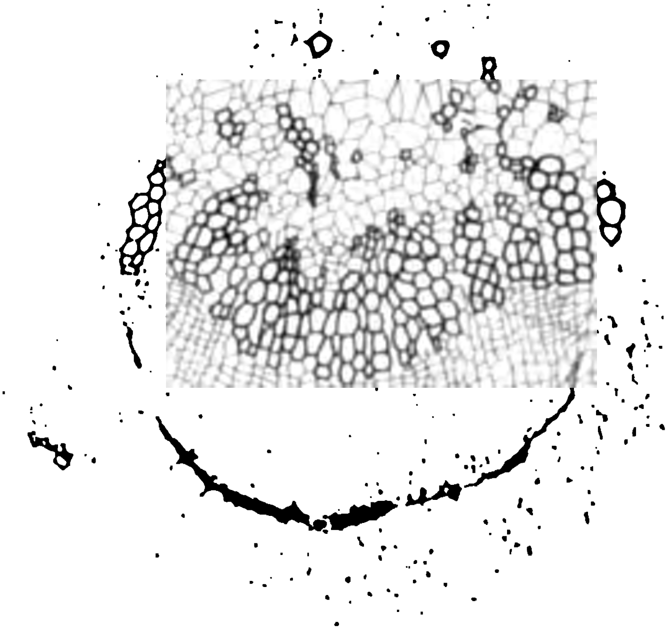


Fig. 6



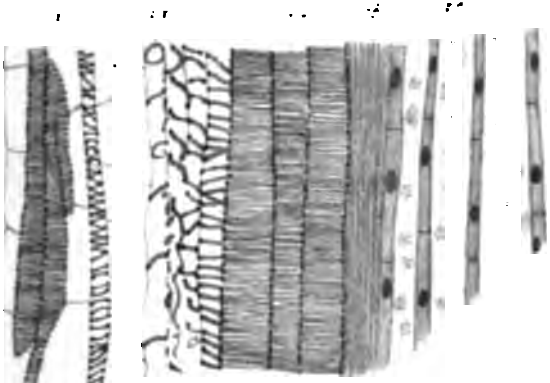


Fig. 7.

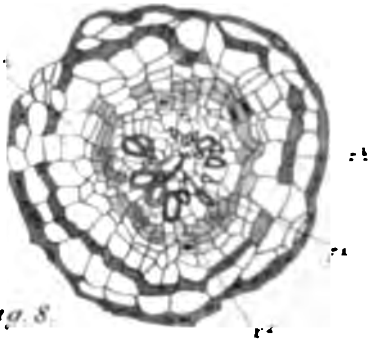
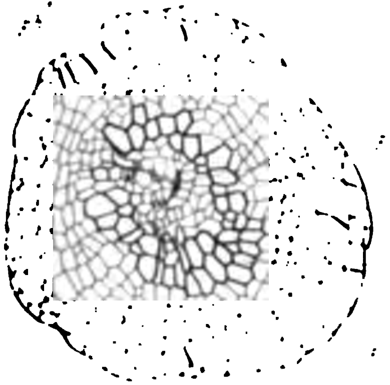


Fig. 8.

Fig. 9.



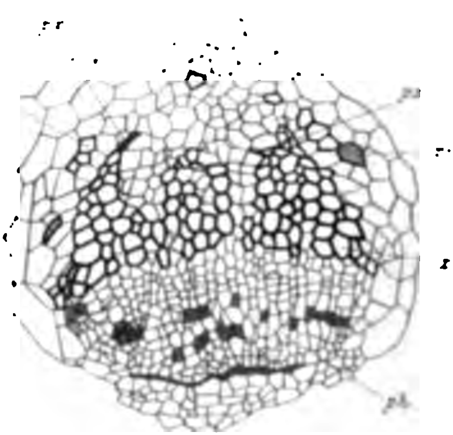


Fig. 10.

Fig. 11.

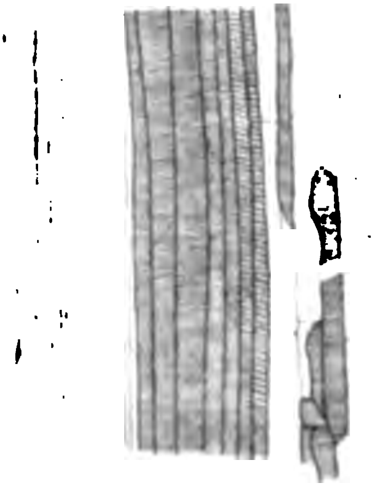


Fig. 12.

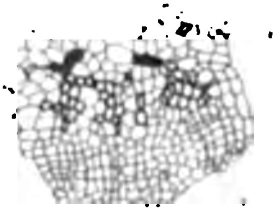
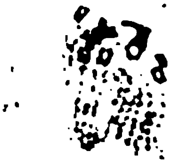


Fig. 13.



SCOTT. — PEDUNCLE OF CYCADACEÆ.

Studies in the Development and Morphology of Cycadean Sporangia:

I. The Microsporangia of Stangeria paradoxa.

BY

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With Plate XXII.

THE especial interest which attaches itself to the Cycadaceae as forms occupying an intermediate position between Phanerogams and Cryptogams has been dwelt on by several investigators who have studied these plants, and has recently been increased by the discovery of zoidiogamic fertilization in one genus (*Cycas*)[1], and of striking anatomical resemblances in the vegetative organs between another genus (*Stangeria*)[2] and the long extinct group of Lyginodendreae.

These considerations justify a fuller examination and account of the existing genera of Cycads than would be necessary in the case of plants of less importance from the point of view of descent: this may provide a basis for comparison of the living representatives of the group among themselves, and with such fossil remains of related plants as may be discovered. The need of this from the palaeobotanical side has been emphasized by Seward[3].

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[1] Ikeno, Bot. Centralbl., 1897, p. 1.

[2] Williamson and Scott, on *Lyginodendron* and *Heterangium*, Phil. Trans. Roy. Soc., 1895, p. 768. See also Scott on Peduncle of Cycadaceae, in the present number of the Annals of Botany.

[3] Science Progress, N. S., Vol. 1, p. 116.

[Annals of Botany. Vol. XI. No. XLIII. September, 1897.]

In these studies the attempt will be made to examine and compare the development and mature structure of the sporangia of those Cycads that have not been fully investigated, so far as material can be obtained. The difficulty of obtaining suitable material for developmental work is very great in plants of this group; but the magnificent collection of Cycads in cultivation at the Royal Gardens, Kew, which in many cases bear cones freely, affords a peculiarly favourable opportunity for gradually accumulating material of the various stages. This investigation was commenced at the suggestion of Dr. D. H. Scott in the Jodrell Laboratory at Kew, upon material already collected by him: and this material was supplemented with further stages as opportunity offered. In connexion with the collecting of material, I wish to acknowledge my indebtedness to the authorities of the Royal Gardens, who, whenever possible, readily gave me permission to preserve the cones which were produced on the different species. The removal of young cones without inflicting injury on the plants is an operation that requires great care, and in this and other ways Mr. Watson kindly assisted me.

The work was commenced under the guidance of Dr. Scott in the Jodrell Laboratory, and has been continued at Glasgow University. To Dr. Scott and Dr. Bower I wish to express my thanks for numerous suggestions and other valuable assistance in the course of the investigation.

Two courses are open in recording the results obtained. The observations made on the considerable amount of material of a number of genera might be collected together, and supplemented when the stages which were wanting could be obtained; or the publication of the results on each genus might be deferred until a fairly complete account of the development of its sporangia was possible. The latter course has many advantages, and will be followed.

The present paper deals with the development and structure of the microsporangia of *Stangeria paradoxa*, Th. Moore. Since it has not been possible to obtain the earliest stages of the development of the ovule, the full account of its development and structure is deferred in the hope of obtaining cones of the required age.

The resemblance which *Stangeria* presents in general habit to a Fern is striking, and has been frequently referred to. In the light of other facts, which make the origin of the Cycads from Fern-like ancestors probable, considerable importance may be attached to this; and it is possible that, in the appearance of the vegetative organs, *Stangeria* presents primitive and not secondarily acquired characters. The fact that structural peculiarities of morphological importance, which in other Cycads are confined to the leaves, have been found in the peduncles of both male and female cones of this species[1], made it a matter of some interest to ascertain whether *Stangeria* differs from other Cycads in the structure and development of its sporangia.

The material was obtained from a large plant, growing in the *Victoria regia* house at Kew, which produces numerous male cones. A series of these, from the first appearance of the tip of the cone among the scale-leaves covering the apex, to the mature cone with dehiscing sporangia, was collected.

The mature sporophylls[2] are inserted on the axis of the cone by short stalks. Each consists of a horizontal portion, the lower surface of which is closely covered with sporangia, and which is continued into the scale-like terminal part. This is clothed with hairs on its outer surface, and is almost vertical, overlapping the sporophylls immediately above, and affording efficient protection to the sporangia during their development. A slight depression in the outer margin of the area covered with sporangia indicates its origin by the junction of two lateral groups of sori. The sporangia differ somewhat from one another in size and shape. A row of large oval sporangia can always be distinguished at the outer margin, and within this is a similar but less regular row; these two rows of sporangia correspond to the outer row of sori. The other sporangia are slightly smaller, and are arranged in sori,

[1] [illegible]

[2] [illegible] Mag. [illegible]

which consist of two to seven sporangia. These sori are difficult to detect in the mature cone, but sometimes the direction of the long axes of the crowded sporangia and of their lines of dehiscence, which run radially in the more regular sori, enable their limits to be determined. As serial sections in a plane parallel to the surface of the sporophyll show, the stalks of the sporangia composing a sorus converge as the surface of the sporophyll is approached. In the intervals between the sori hairs are present, while they are not found between the individual sporangia of a sorus. Each sporophyll bears a large number of sporangia[1]; these are less numerous on the scales near the apex of the cone, but their arrangement is the same.

The youngest cones that could be obtained without risk of injury to the plant were too far advanced to enable any observations on the development of the sporophylls to be made; they measured 28 mm. in length, exclusive of the peduncle. The early stages of development of the sori and sporangia could, however, be followed in cones of this size, for not only were the sporophylls in the apical region in a less advanced stage of development than those inserted nearer the base, but sori of different ages were present on the same sporophyll.

The young sporophyll (Fig. 1) has a short stalk. The horizontal soriferous portion is distinguishable by its white glistening appearance from the hairy surface of the terminal scale-like part, which extends as a V-shaped projection between the two lobes of which the horizontal portion consists, but does not completely separate them. The sori in the youngest scales examined formed a slightly curved row on each side, immediately within which was a group of sori differing in size from one another. The outer row of sori gives rise to the two outer rows of sporangia, while the inner group consists of the first developed sori of more central sporangia. These are seen to be distinct from one another in older scales

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[1] According to Al. Braun more than 100 (Die Frage nach der Gymnospermie der Cycadeen: Monatsber. d. K. Akad. d. Wiss., Berlin, 1875, p. 343, note).

(Fig. 2). The part bearing the sori has increased in size, and its lateral lobes are more prominent. Sori continue to arise toward the outer border of the lateral lobes, and also on the elevated median ridge. As the latter increase in number, they connect the two groups of sori which were at first separated by a considerable interval. Those last formed are the most regular in form; they arise as circular elevations, the margins of which soon become distinct, and they are at first separated from one another by wider intervals than the earlier formed sori. The appearance presented by the latter (Fig. 1) suggests an origin by unequal growth of a slightly elevated central region.

The differentiation of the sorus into sporangia agrees closely with what takes place in *Ceratozamia*. On the same sporophyll all stages from the first indication of a sorus to sporangia of considerable size are present (Figs. 2, 3), while Warming's figures suggest that the numerous sori on any sporophyll of *Ceratozamia* are at the same stage in their development[1].

One peculiar feature in the relation of the individual sporangia of a sorus to each other is best illustrated by a transverse[2] section (Fig. 4). In many cases the sporangia become equally distinct from one another at an early stage, but sori were frequently observed, in which some or all of the sporangia remained for a time united in pairs. In the sorus figured three such pairs are seen, the crosses indicating the position of the sporogenous groups of cells, which were similar to that shown in Fig. 8.

The superficial cells of the region of the sporophyll, on which sori are about to arise, have larger nuclei and more abundant protoplasm than the underlying ones. The epidermis is a perfectly definite layer dividing only by anticlinal walls. The sorus appears as a slight elevation, which is at first flat-topped, but at an early stage becomes depressed centrally by the more rapid growth of the surrounding cells. The origin of the sorus depends, partly on the increase in size of the cells of the hypodermal layer, and partly on division and growth of the cells underlying this. Sometimes the latter owe their origin to earlier divisions of the hypodermal layer, in which case a section of the sorus shows vertical rows of cells; in other sections this is not so obvious. The merismatic appearance of the tissue extends more deeply beneath a developing sorus than elsewhere, and in a slightly later stage is seen to be continuous with the procambial strand of the bundle, which terminates beneath each sorus. In radial sections of such young sori certain hypodermal cells of large size and with prominent nuclei can be recognized. They occupy a definite position, about midway between the depressed centre and the margin. Usually two such cells are visible in a vertical section passing through one of the young sporangia (Fig. 5), but comparison with the adjoining sections of the series and with transverse sections of sori of similar age shows that a group of four cells is present, only two of which can appear in a vertical section. From this group of cells the sporogenous tissue is derived. The frequency with which anticlinal divisions take place in the hypodermal layer renders it a matter of great difficulty to determine whether this group is derived from a single cell, as Goebel[1] suggested in the case of *Zamia*, or not. In certain cases I am of opinion, from a careful study of the arrangement of the cell-walls, that this is not the case, but in a few examples it appeared to be a possible interpretation. No single cell could, however, be distinguished as occupying the position of the cell-group in younger sori.

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[1] Warming, Contribution à l'histoire naturelle des Cycadées, Copenhagen, 1879. Tab. V, Figs. 3, 4, 8, 9, 10.

[2] By a *transverse section* of a sorus or sporangium is meant one parallel to the surface of the sporophyll. A section perpendicular to the surface of the sporophyll will be spoken of as a *vertical section* and, when possible, will be distinguished as *radial* or *tangential* with reference to the axis.

Each of the four cells undergoes division by a periclinal wall into a flat outer cell and an inner one of slightly greater size (Fig. 6). From the four inner cells thus produced the sporogenous tissue is derived. Their origin is similar to that

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[1] Goebel, Vergleich. Entwicklungsgesch. d. Pflanzenorgane, p. 395.

of the archesporial cells in the pollen-sac of an Angiosperm, while the outer segments correspond to the primary tapetal cells.

The first division in the sporogenous cells is by a periclinal wall, so that a group of four cells is seen in a vertical section (Fig. 7). Since four sporogenous cells are visible in transverse sections of sporangia of this age (Fig. 8) it is clear that there is a cubical group of eight cells. The cells surrounding each sporogenous group keep pace with its increase in size and undergo frequent divisions. At the central point of the sorus and between the developing sporangia the hypodermal cells soon lose their merismatic character and cease to grow, so that the sporangia become distinct from one another. In such cases as that shown in Fig. 4, growth has not been arrested in the interval between the sporangia of each pair, but these also become free from one another at a later stage. The stalk of the sporangium is derived from the growth of the tissue beneath the sporogenous group.

After the stage represented in Fig. 7 has been reached, the divisions in the sporogenous cells do not follow in exactly the same order in different sporangia. Usually each of the upper cells is divided by anticlinal walls, and the resulting cells undergo periclinal divisions. From the lower of the shaded cells seen in Fig. 7 the greater part of the sporogenous tissue originates: these cells divide, in the more regular examples, into four by periclinal and anticlinal walls. The subsequent divisions of these cells occur in various directions, but the position of the earlier division walls remains traceable for some time (Figs. 9, 10). In transverse section the whole of the sporogenous mass can as a rule be referred to the group of four cells present in similar sections of younger sporangia (Fig. 11).

The shape of the sporogenous group differs to a considerable extent in sporangia of the same age, as will be seen on comparing Figs. 9 and 1[illegible]. These differences stand in relation with the general form of the sporangium, which appears to be influenced in no small degree by the pressure to which it is subjected during development. That the pressure is considerable is indicated by the manner in which the upper surface of the sporophyll becomes marked by small depressions corresponding to the sporangia of the sporophyll above. When this pressure acts upon a sporangium, the form assumed at this stage is short and broad, the sporogenous tissue is extended parallel to the flat top of the sporangium, and the cells of the wall which owe their origin to the primary tapetal cells are flattened (Fig. 9). It sometimes happens, however, that by the presence of large sporangia around, or by the margin of the sporophyll becoming turned down to form a sort of rim, the sporangium is almost relieved from pressure in the vertical direction. It then assumes the form shown in Fig. 10, where the sporogenous tissue forms an elongated square prism retaining the general proportions seen in Fig. 7; the general form of the sporangium is correspondingly elongated, and the cells of the wall derived from the primary tapetal layer have not the flattened appearance seen in the former example.

The sporangium increases considerably in size without any further differentiation becoming apparent. A large mass of sporogenous tissue is formed by repeated divisions, and the wall increases in thickness (Fig. 12). The thickness of the wall is usually greater on the side toward the centre of the sorus and the rectangular outline of the upper part of the vertical section is due to its thickness at the angles. At the sides it consists of four or five layers of cells. At this stage the epidermal cells are still thin-walled. The cells adjoining the sporogenous mass usually divide by walls parallel to the limits of the latter, and the more or less complete double layer thus produced is distinguishable even in the mature sporangium. This layer, the further development of which will be described below, forms a convenient indication of the limit between the wall and the sporogenous tissue.

Until this stage (Fig. 12) the tapetum is not recognizable, but shortly after the divisions in the inner layer of the wall its origin can be traced. It arises from the sporogenous tissue by the cutting off of tabular cells from the outer layer or, less commonly, by the direct conversion of the outer cells into cells of the tapetum. Fig. 13 shows the tapetal layer at this stage, and demonstrates, both by the arrangement of the cell-walls and by its position with regard to the double layer of cells of the wall, that it has arisen from sporogenous cells. Usually the tapetum is a single layer, but it may become at places two or even three cells thick, by the deeper cells of the sporogenous mass assuming the characters of tapetal cells. Its cells at first resemble those of the sporogenous tissue so closely that care is necessary to distinguish them, but changes soon occur in the latter which render their distinction an easy matter, and, by comparison with earlier stages, place their mode of origin beyond doubt. The sporogenous cells are now the mother-cells of the pollen-grains, and their walls undergo a change which causes them to stain deeply with Bismarck-brown, while the walls of the tabular tapetal cells remain thin and are more difficult to distinguish than before. Comparison of a large number of sporangia shows that the mode of origin of the tapetal layer described above is the ordinary one in *Stangeria*; but I am not prepared to state that this layer is never derived in part from cells adjoining the sporogenous tissue, although no sporangium in which this was clearly the case has been found.

Immediately outside the tapetum the double layer of cells can in most cases be recognized. Its cells are usually flattened and contain elongated nuclei which appear homogeneous and highly refractive. Between these cells and the epidermis, the cell-walls of which are becoming thickened, the remaining layers of the wall are present. Their nuclei as a rule do not take on the same highly refractive appearance as those of the double layer of cells.

Some of the spore-mother-cells do not develop further, but undergo a change closely resembling that described by Treub[1] in *Zamia*, both nucleus and cytoplasm becoming highly refractive and undistinguishable from one another; these cells stain deeply and uniformly (Fig. 14). The number of cells that undergo this sterilization is not great, but the fact of its occurrence is of interest, for comparison with what takes place in the large sporangia of Pteridophyta, e.g. *Equisetum*[1], *Psilotum*[2], *Ophioglossum*[3], and in the pollen-sacs of some Angiosperms, e.g. *Arum maculatum*. The sterilized cells are always much smaller than the surrounding sporogenous cells with which they agreed in size before the change commenced. This circumstance, taken together with the fact of the sterilized cells being more or less flattened in most cases, suggests that increase of pressure within the sporangium is at least a factor in their production. It is of interest in regard to this to note that at the time of their appearance the epidermal layer has just acquired its thick walls, which may be presumed to offer greater resistance to the increase in size of the sporogenous mass within. It is possible that deficient nutrition may be another factor in their production, but their frequent occurrence close to the tapetum itself points to the operation of some other cause.

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[1] Recherches sur les Cycadées: Ann. Jard. Buitenzorg, Vol. ii, p. 36, Pl. III, 1, 4, 5.

The spore-mother-cells become isolated from one another. The tetrad-division was not followed in detail, but the tetrads still enclosed in the wall of the mother-cell closely resemble those of other Cycads. Fine granules are present between them, but only in small amount. The spherical spores, when they become free, do not nearly fill the cavity of the sporangium (Fig. 15).

The tapetum persists as a complete layer within the wall of the sporangium; its cell-walls have disappeared. The individual cells stain deeply; each possesses a nucleus which never assumes the highly refractive appearance of the layer immediately outside. The nucleus often breaks up into two or three, apparently by fragmentation.

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[1] Bower, Phil. Trans. 1894, p. 500, Fig. 21, Pl. 43.

[2] Ibid. p. 550, Fig. 143, Pl. 51.

[3] Rostowzew, Recherches sur l'Oph. vulg., note préliminaire, p. 28, Taf. 1, Figs. 1, 5.

The two layers of cells, the origin of which from the cells of the wall adjoining the sporogenous mass was described above, are now so crushed that the limits of the cells are difficult to make out, and the whole appears under low powers as a thin band outside the tapetum (Fig. 15). The cells of this layer stain deeply, and are sharply distinguished from the outer cells of the wall, which have oval nuclei of granular appearance. There are three to six layers of these cells and the wall is bounded by the epidermis, the cells of which differ in form and structure according to the part they play in the dehiscence of the sporangium.

When examined from the surface, the epidermal layer is seen to consist of cells with more or less thickened walls, elongated in the direction parallel to the line of dehiscence. The latter is distinguished by its colourless cell-walls, which contrast with the brown colour of the cells of the rest of the wall. Its cells, which form two rows, are somewhat shorter than those on either side; they have greatly thickened walls in which numerous simple pits are present. The line of dehiscence extends along the whole length of the side of the sporangium which is turned away from the sporophyll; it reaches from close to the stalk almost to the apex, where it is continuous with a group of cells which agree with it in the appearance and structure of their walls, but have more or less circular cavities. These cells which occupy the apex, correspond in position as Warming has stated[1], to what is usually described as the annulus of *Osmunda* or *Angiopteris*. In *Stangeria* they do not project above the level of the surrounding cells though this is the case in some other genera of Cycadaceae[2]. The apex of the sporangium of *Stangeria* corresponds closely with that of *Ceratozamia*. The cells of the rest of the sporangial wall have cell-walls of a yellow colour and of considerable thickness. When viewed from the outside but little difference can be detected between them: the cells near to the line of dehiscence have however, some what thicker walls with more numerous pits than those toward the opposite side of the sporangium. If a section of a sporangium at right angles to the line of dehiscence be examined, the difference is found to be much more considerable than appears in surface view. The cells for some distance on either side of the line of dehiscence have all their walls, especially the inner and vertical, strongly thickened (Fig. 18). Toward the apex these bands of thick-walled cells are broader than near the stalk, and the transition from them to the thinner-walled cells of the lateral region is more gradual. The latter near the apex have thin outer but fairly thick vertical and inner walls, while further from the apex the cells become more uniformly thin-walled.

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[1] Warming, [illegible] Pl. V, Fig. 12.

[2] [illegible] III, Fig. [illegible]

There are thus bands of thick-walled cells on either side of the line of dehiscence, which meet at the apex in the cap of isodiametric thick-walled cells, while the rest of the epidermal layer is composed of comparatively thin-walled cells. The relation of the thin- and thick-walled regions of the wall is closely similar to that existing in *Angiopteris*[1]. The mechanism by which the sporangium opens appears also to be essentially the same[2]. When the cells dry up at maturity, the thin-walled cells of the lateral region contract and pull upon the bands of thick-walled cells, thus widening the slit: this in *Stangeria* is formed by the separation of the two rows of cells which compose the line of dehiscence, while the cells themselves remain intact. The widely gaping slit, which extends from the stalk to the apex, is directed downwards in the natural position of the sporangia. The mature sporangium can be induced to open by drying, and closes again when moistened.

Numerous stomata are present in the epidermis of the side of the sporangium away from the line of dehiscence and near the stalk. Their position is indicated in Fig. 17 by dots on the wall. Their guard-cells are depressed below the surface, and subsidiary cells are present. The stomata do not become fully developed until the sporangium is nearly mature. They are found in a similar position on microsporangia of *Ceratozamia* and *Encephalartos*, the only genera of which mature material was at hand for comparison. Stomata are present on the ovules and sometimes the pollen-sacs of Angiosperms, but, so far as I am aware, not on the sporangia of any Vascular Cryptogam. The two sporangia represented in Fig. 17 also illustrate the difference in form between the sporangia at the outer border of the soriferous region (*a*) and those situated more centrally (*b*); the existence of this difference was referred to above, and the figure will explain its nature without further description.

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[1] Zeiller, Gîtes Minéraux, Flore fossile, p. 18, Fig. 13.

[2] The mechanism of dehiscence in *Angiopteris* is described in a paper by Bower, read before the Royal Society on May 27, 1897, but not yet published in full. I am indebted to Dr. Bower for placing his results and preparations at my disposal for purposes of comparison with *Stangeria*.

The *pollen-grain* of *Stangeria*[1] is at first spherical; later one side becomes infolded, and its shape is oval. At first only one nucleus is present (Fig. 16, *p*), but before the pollen is shed each grain contains three cells, the large vegetative cell with its spherical nucleus and two cells with thin cell-walls (Fig. 18); these are situated against the side which becomes infolded. Since only these two cells were present in pollen which had been germinated by Dr. Scott, it is safe to conclude that they are the only ones cut off in the course of development of the pollen of *Stangeria*. *Stangeria* thus agrees with the other Cycads in the structure of its pollen.

The *ovule* of *Stangeria* agrees closely in the later stages of development with the Cycads examined by Warming[2] and Treub[3]. The embryo-sac was already present in the youngest material yet obtained; it is developed from the lowest of a row of three cells, which is derived from a single cell occupying a central position in the nucellus. The embryo-sac, in the ovules of this cone, was but slightly larger than the other cells of the row, and contained a single nucleus. It increases rapidly in size, and its nucleus gives rise to a considerable number of daughter-nuclei, which are situated in a layer of protoplasm lining the wall. In a further stage the embryo-sac is filled with the thin-walled tissue of the prothallus; its wall is thick and cuticularized. Surrounding the large embryo-sac in this advanced stage is a layer, one to three cells in thickness, which is all that remains of the sporogenous tissue. This layer appears to be of the nature of a tapetal layer. It persists although the cells outside it are becoming crushed as the embryo-sac increases in size, and its cells, which are frequently elongated vertically to the wall of the latter, contain in many cases two or three nuclei. If derived, as seems probable, from the outermost cells of the sporogenous mass, its place of origin would resemble that of the tapetum in the microsporangium. Further observations are required on this and other points, but the brief account given above will serve to show that *Stangeria* agrees closely with *Ceratozamia* or *Cycas* in the development of the embryo-sac.

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[1] Braun, loc. cit. p. 315, described the pollen-grain of *Stangeria*, but did not determine the number of basal cells (Basalzellen).

[2] Recherches et remarques sur les Cycadées. Copenhagen, 1877.

[3] Loc. cit.

SUMMARY.

1. The microsporangia are arranged in sori, the development of which is similar to *Ceratozamia:* sometimes the sporangia remain for some time united in pairs.

2. The sporogenous cells, usually four in number, are derived by periclinal division from cells of the sub-epidermal layer. The superficial cells take no part in their formation.

3. The tapetum arises, when the sporangium has attained a considerable size, from the outer cells of the sporogenous mass. Previously the inner layer of cells of the wall has given rise to a double layer of cells which assume peculiar characters.

4. Isolated cells throughout the sporogenous mass undergo sterilization.

5. The structure of the epidermal layer of the wall and the

mechanism of dehiscence present close resemblances to *Angiopteris*. Stomata are found on the sporangium.

6. In the pollen-grain two cells are cut off from the large vegetative cell before germination.

7. In the later stages of development the ovule resembles other Cycads.

CONCLUSION.

Several observers[1] have discussed the extent to which the Cycadaceae agree in the development and structure of their sporangia with the Marattiaceae on the one hand, on the other with Angiosperms. It will therefore be unnecessary to do more than point out in what respects *Stangeria* shows resemblances to these two groups, deferring a more detailed treatment of the subject until a larger body of facts has been accumulated. In this paper the microsporangia alone will be considered.

The arrangement of the sporangia in sori and the mode of development of the latter agrees closely with *Angiopteris*, and this has rendered the terms sporangia and sorus more suitable than those applied to the parts of the stamen of Angiosperms. On the other hand the origin of the sporogenous cells from the hypodermal layer a distinct epidermis being present, is quite different from what takes place in any Vascular Cryptogam hitherto investigated, and except for the small number of sporogenous cells, is similar to their origin in the pollen-sac of an Angiosperm. In the present state of our knowledge of the details of the apical meristems of Cycads it is impossible to properly estimate the weight to be attached to this. If, as seems probable, the apical meristem is stratified[2], no single initial cell being present, the general agreement which has been found to exist between this mode of arrangement of the formative tissues and the sub-epidermal position of the sporogenous cells[1] would tend to diminish the importance to be attached to this difference between Marattiaceae and Cycadaceae. It is to be noted that, though the stratification of the apical meristem was imperfect in the Cycads investigated, the stratification is perfectly definite in the young sporophyll, the epidermis never undergoing periclinal divisions.

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[1] Treub, W[illegible] [illegible]

[2] Strasburger, [illegible] Coniferen [illegible] Taf. XXX, Fig. 38.

The general agreement between the sporangia of these groups lends weight to this mode of regarding the question. The resemblance in general form and appearance between the sporangia of Cycads and Marattiaceae has been noted especially by Braun and Warming. There are two points, however, which deserve further notice, the origin of the tapetum, and the mode of dehiscence of the sporangium.

In the Marattiaceae the tapetum arises normally from the layer of the wall adjoining the sporogenous cells, and, on theoretical grounds, a similar origin might have been expected in the bulky sporangia of *Stangeria*. The case has been shown to be more complex, however. Three layers of cells around the mother-cells of the pollen-grains are found to have undergone special changes. The innermost layer is derived from the sporogenous mass, while the other two layers arise from the cells of the wall adjoining it. Since only the inner layer takes on tapetal characters, the tapetum has been described as arising from sporogenous cells. But the layers outside, which doubtless contribute to the nutrition of the spore-mother-cells, may possibly *represent* a tapetum derived from cells surrounding the sporogenous group, an additional tapetal layer having arisen from the latter. This explanation becomes more likely when it is borne in mind that other cells throughout the sporogenous mass lose the function of spore-production, and their substance aids in the nourishment of the rest. The difference in appearance of the tapetum from the isolated sterilized cells may be connected with the additional function it performs in virtue of its peripheral position. This view is only advanced as a possible way of explaining what must be regarded as an important difference between the sporangia of Marattiaceae and Cycadaceae.

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[1] Bower, Studies in the Morphology of Spore-producing Members: Ophioglossaceae, p. 7.

The similarity in the mode of dehiscence is a necessary result of the similar development of the epidermal layer. It is interesting on account of the close agreement between *Stangeria* and *Angiopteris* in all the essential features of the mechanism, and on this account is entitled to some weight. But, since there is no evidence to show whether the presumable Fern-like ancestors of the Cycadaceae possessed separate or incompletely separated sporangia, it is equally possible that these resemblances afford rather a case of parallel development.

The general conclusion so far as the results recorded above enable it to be stated is *that* Stangeria *shows no characters in the development and structure of its sporangia which can be regarded as primitive in any further sense than is the case for other genera of the Cycadaceae.* Some details, probably common to the other Cycads, appear to be shown more clearly in this genus.

EXPLANATION OF FIGURES IN PLATE XXII.

Illustrating Mr. W. H. Lang's paper on *Stangeria.*

Fig. 1. Under surface of the horizontal portion of a young sporophyll: a group of young sori is present on either side of the middle line. Slightly magnified.

Fig. 2. Similar view of an older sporophyll. The earlier formed sori show separate sporangia: sori are still in course of development near the middle line and towards the margin of the sporophyll. Slightly magnified.

Fig. 3. A group of sori from a sporophyll of the same age as Fig. 2, more highly magnified. To the right is a young sorus in which no indication of the sporangia is apparent.

Fig. 4. Transverse section of a young sorus; the sporangia are united in pairs. x = portion of sporogenous cells. × 80.

Fig. 5. Part of a vertical (radial) section through a very young sorus, showing a single sporangium : c = the central point of the sorus; $x\ x$ = cells from which the sporogenous cells will be derived. × 400.

Fig. 6. Vertical (radial) section of a slightly older sorus, which shows two sporangia in median section: the sporogenous cells are shaded. × 400.

Fig. 7. Vertical section of an older sporangium, showing the first periclinal division of the sporogenous cells, which are shaded. × 400.

Fig. 8. Part of a transverse section of a sorus. The figure shows a single sporangium of about the same age as that in Fig. 7: the sporogenous group still consists of four cells (shaded in the figure) as seen in transverse section. × 400.

Figs. 9, 10. Vertical (radial) sections of older sporangia; the sporogenous masses are shaded. These figures illustrate the difference in form that exists between sporangia of the same age. × 400.

Fig. 11. Transverse section of a sporangium of similar age to Fig. 9 or 10: the sporogenous tissue, which is shaded, can be referred to the four cells shown in Fig. 8. × 400.

Fig. 12. Vertical radial section of an older sporangium just before the tapetum becomes recognizable: the sporogenous cells are shaded. × 300.

Fig. 13. Part of a similar section of a slightly older sporangium, showing the origin of the tapetum from the outer cells of the sporogenous group. *t* = tapetum, the cells of which are shaded; *sp* = sporogenous cells; *w* = inner part of sporangium-wall. × 400.

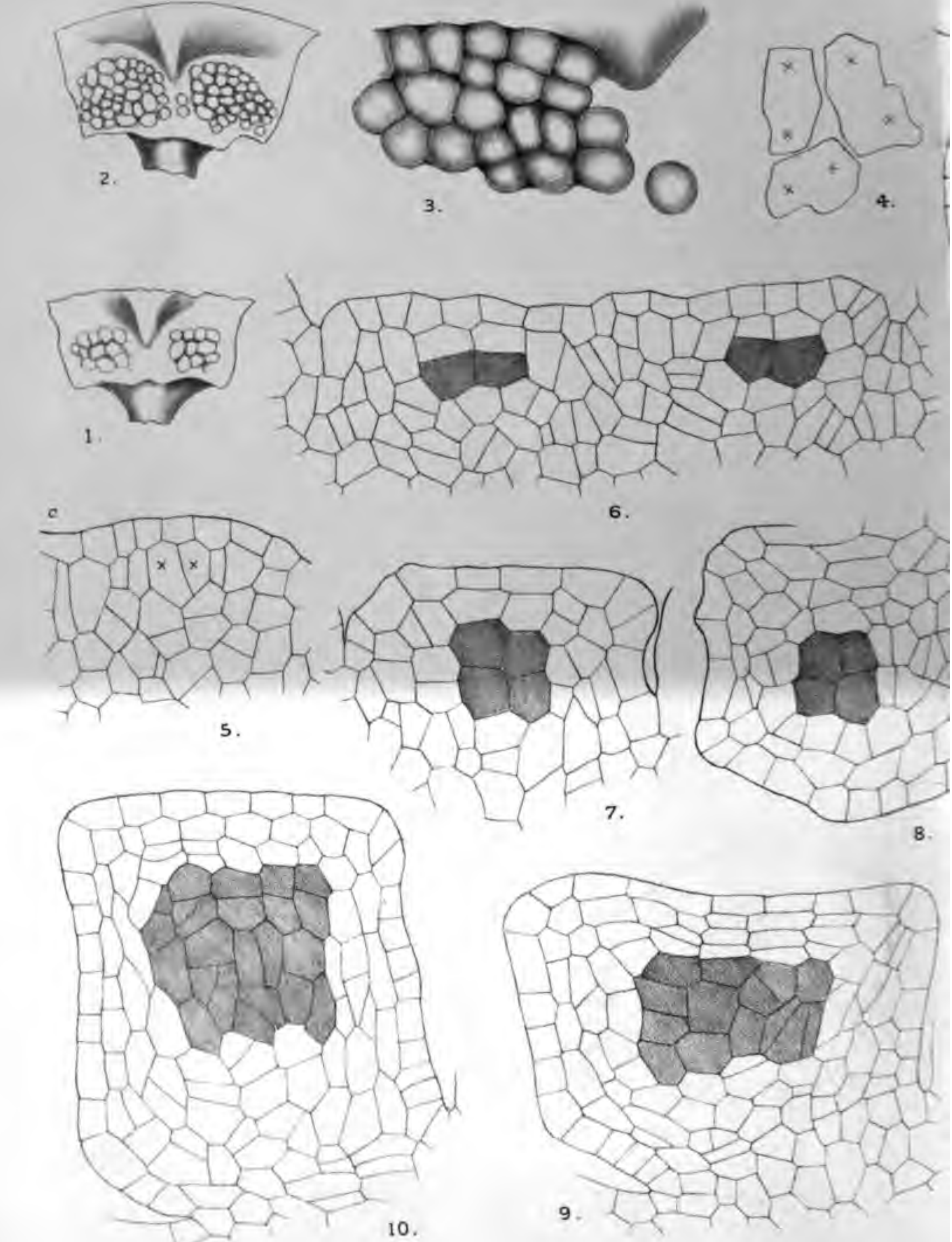
Fig. 14. Spore-mother-cells, one of which is undergoing sterilization. × 550.

Fig. 15. Vertical, nearly radial, section of a sporangium containing isolated pollen-grains. The tapetum is still present as a complete layer of cells around the cavity: the cells of the wall immediately outside it are crushed: *t* = tapetum. × 100.

Fig. 16. Part of a vertical (tangential) section through a sporangium of the same age as Fig. 15, including the line of dehiscence. *e* = epidermal layer of thick-walled cells; *t* = tapetum; *p* = pollen-grains; *d* = line of dehiscence. × 300.

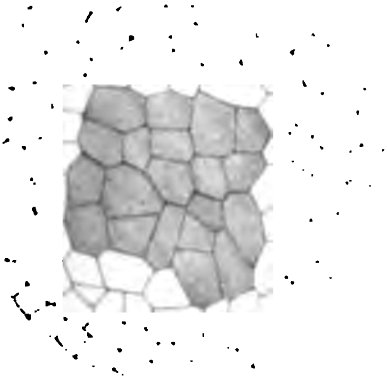
Fig. 17. Side view of two mature sporangia: *a*, from the outer row of large sporangia; *b*, from the central sporangia. The dots indicate the position of stomata. *x x* = position of line of dehiscence. × 6.

Fig. 18. Ripe pollen-grain, showing the vegetative cell and the group of two cells. × 1000.



W.H.Lang, del.

LANG.— STANGERIA PARADOXA.



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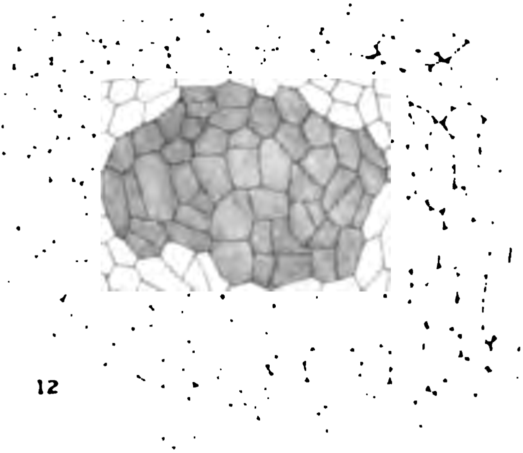

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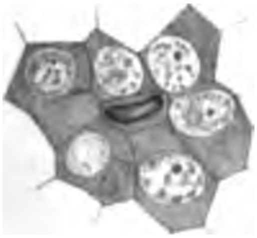

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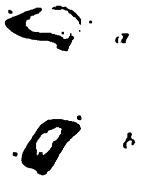

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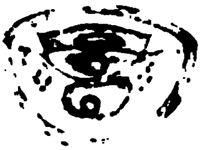

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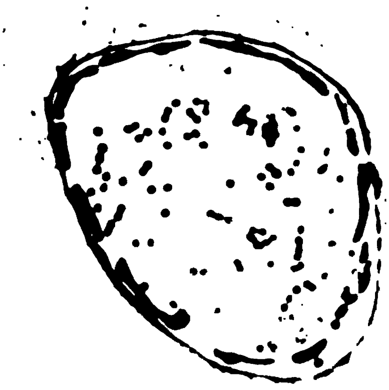

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The Effects of Tropical Insolation.

BY

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IN a previous paper[1] an account has been given of the phenomena of assimilatory inhibition by insolation in the temperate regions, in which it was shown that most plants are resistant to ordinary exposure; but that, in the leaves of certain Phanerogams and shade-loving plants, by prolonged exposure to perpendicular illumination, a generally temporary stoppage of CO_2-assimilation, with or without an accompanying more or less complete decolourization of the chlorophyll-corpuscles, might be produced, whilst in certain Algae, *Chara*, &c., relatively short exposure might readily cause a stoppage of assimilation, generally accompanied, however, by a marked decolourization of the chlorophyll-corpuscles, and if at all prolonged being followed first by the death of the chlorophyll-grains of the part exposed, and finally by the death of the part exposed *in toto*[2].

Advantage was taken of a stay in the tropics at Buitenzorg, Java, to corroborate and extend these observations; whilst, during a short stay in Ceylon, a number of accessory and

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[1] Assimilatory Inhibition: Journal of the Linnean Society, 1896.

[2] Loc. cit. p. 439; and Journal L. S., Vol. XXXI, 1897, p. 573.

[Annals of Botany, Vol. XI. No. XLIII. September, 1897.]

confirmatory observations were made. The methods employed were the same as those already described in previous papers, the evolution or non-evolution of oxygen, as detected by the Bacterium-method, under optimal illumination, being taken as a test for the presence or absence of the power of assimilation.

It might at first sight appear probable that under the intense tropical sunlight all fully exposed green leaves should suffer considerably; but, as a matter of fact, so long as the plant is abundantly supplied with water and is kept cool by transpiration, it suffers no more under such exposure than do plants in temperate regions. It may indeed be concluded, with a fair amount of certainty, that practically in all tropical plants which normally grow in fully exposed situations, provided the leaves are healthy and abundantly supplied with water, no assimilatory inhibition directly due to insolation ever takes place under normal conditions of exposure. Thus the healthy adult leaves of the following plants, after a full day's exposure to perpendicular solar illumination, were green, fresh, and showed an active power of assimilation: *Caryota maxima*, Bl., *Musa Ensete*, *Kentia McArthuri*, *Alocasia indica*, Schott., *Artabotrys Blumei*, *Uncaria sclerophylla*, and *Areca Catechu*, L.; whilst in *Bauhinia*, *Caesalpinia*, *Acacia*, and *Dalbergia linga*, if the leaves, owing to their paraheliotropic irritability, are allowed to droop or fold together, no appreciable effect is produced; but if they are kept fully exposed to perpendicular illumination they become much paler green in colour, are more or less wilted, and the power of assimilation is weakened, diminished, or inhibited, temporarily or permanently.

There are a variety of ways in which the leaves of exposed tropical plants may be rendered capable of withstanding excessive insolation. Perhaps the commonest, though the least obvious, is by means of some invisible inherent organic change or difference in the chlorophyll-bodies of plants growing in exposed situations, as compared with those frequenting shady habitats. Along with this go changes in the shape and position of the palisade-parenchyma layers, and in the thickness, shape, and transparency of the epidermis and cuticle, adding to the protection afforded by the apostrophic position assumed by the chlorophyll-grains of the palisade-parenchyma when the illumination is intense.

Another important mode of protection is by the folding or sinking of the leaves or leaflets when exposed to strong illumination, so that their surfaces are parallel to the incident ray. In leaflets which fold together and overlap one another, and perhaps also to a slight extent in leaves or leaflets which simply twist on their axes, as a secondary consequence of the change of position, the amount of transpiration is more or less markedly diminished, and it is quite possible that in certain cases this may be an important function of the paraheliotropic movement.

Finally, the presence of a red pigment in the leaves of many plants must shield them to a certain extent from the effects of too intense and prolonged illumination.

It must be remembered that the photochemical intensity of the tropical sun is by no means so great as might be supposed, and this is especially the case in moist, damp climates, such as those of Buitenzorg (W. Java) and Peradeniya (S. W. Ceylon), in which the moisture causes an absorption more especially of the photochemical rays. In a recent research Wiesner[1] has shown that the greatest chemical light-intensity observed at Vienna was 1·5 units, and at Buitenzorg not more than 1·612 units, whilst the daily total amount in Buitenzorg during November and December is equal to that in August in Vienna, and the total amount daily in January at Buitenzorg is equal to that in June in Vienna, the greater intensity of the tropical light being counterbalanced by the shorter day.

In several more or less shade-loving tropical plants, however, prolonged exposure to perpendicular illumination[2] produces a distinct effect upon the power of assimilation which may, if the leaf be sensitive and the exposure very severe and prolonged, result in a permanent stoppage of assimilation, followed by the death of the leaf, wholly or in parts only. In all cases, after prolonged exposure, the leaf is paler than normal, and may become browned (*Cocculus Beccari*, &c.), or in some cases almost colourless (greenish or yellowish white, *Pisonia*, *Selaginella*). After being examined, the plants are kept well shaded, and from time to time fresh portions of the still attached leaves are examined, this method being necessarily adopted on account of the difficulty of obtaining perfect recovery in isolated parts.

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[1] Wiesner, Untersuchung über das Photochemische Klima von Wien, Buitenzorg, und Kairo: K. Akad. d. Wiss. in Wien, July, 1896.

[2] The necessary daily periods of uninterrupted sunlight were obtained in Java

Cocculus Beccari. Exposed from 6 a.m. to 4 p.m. Many leaves, especially the older ones, more or less yellowish or brownish entirely or in patches. Young adult leaves show weak assimilation, chiefly in spongy mesophyll, older leaves none or very weak. In one day assimilation active in young leaves, in older leaves weak to moderately active, in yet others not perceptible, though leaves have not become perceptibly greener. After full exposure for three to four days, many older leaves are quite yellow, younger leaves green in parts and show none or very weak assimilation. Next day weak to moderately active assimilation in younger leaves, older yellowish leaves none. Of latter many do not recover, although they may remain living for several days, the younger leaves finally become almost without exception quite green and normal again.

Pisonia alba, Sparr. Plant exposed for several days. Leaves from almost quite white to yellow or yellowish green: latter faint to moderately active assimilation. After thirty hours in faint diffused daylight, former leaves faintly green and show weak assimilation. In three to four days leaves all deep green to yellowish green and assimilation active. In the quite white leaves the chlorophyll-grains are almost colourless and difficult to distinguish. Such leaves do not become

towards the close of the dry season of 1896 and during various cloudless days during the wet season of 1896–7, the S. W. monsoon of this period being unusually well favoured in this respect. A few, chiefly physical and biological, observations, were made in Ceylon during the dry period in the spring of 1897 between the two monsoons.

fully green for from seven to ten days. Young but adult leaves exposed for ten hours to perpendicular rays are pale green to yellowish green, assimilation absent or faint in parts. Next day leaves slightly greener, assimilation weak to moderately active. Similar leaves exposed, but layer of cold water interposed, show the same colour as before, assimilation absent, or in parts weak to moderately active; in some leaves weak assimilation in all parts: recovery as before.

Dendrobium crumenatum, Lindl. Plants exposed to perpendicular rays from 6 a.m. to 4 p.m., assimilation weak or absent; leaves slightly paler and yield weaker chlorophyll-extract than equal weight of shaded leaves. If part of one leaf is covered by paper or tinfoil, at end of exposure it is distinctly fresher and greener, and shows moderately active assimilation, rest of leaf weak or none. Next day nearly all leaves are living, normal; assimilation is moderate to quite active. In a few leaves assimilation is weak or in parts absent, these leaves in a few days turn yellow and die.

Selaginella sp.? Exposed for some days, leaves turn quite pale, and become when shaded green again. One day's exposure causes a distinct fading; and many leaves on the exposed side, though green to pale green or yellowish green, show weak or no assimilation in parts or entirely. If the half of the leaf only is used, the corresponding half next morning may show a fairly active power of assimilation.

Drymoglossum piloselloides, Prsl. After ten hours, exposed vegetative leaves slightly paler; chloroplastids normal and with abundance of starch; assimilation weak or absent; in partially exposed leaves with similar abundance of starch, fairly active. Next day assimilation moderate to quite active, but in a few leaves none. The latter may in some cases remain living for several days without regaining the power to assimilate; they slowly turn pale yellow or brownish yellow, and finally die. A weak power of assimilation may return to the preparations previously examined if they are kept in a drop of water in a damp chamber.

Adiantum cuneatum, Lug. and Fisch. and *A. assimile*, Sw. After six hours' exposure, assimilation faint or absent. Leaves paler and some may not recover; the rest in one day show fairly active assimilation.

A. rhodophyllum. Leaves thicker, stouter, and more resistant. After eight hours the leaves are paler than normal, and show weak, or in some cases almost imperceptible assimilation; next day active again. If the leaves are old, assimilation may be absent after eight hours' exposure and not return. Young leaves are red till half grown, and show normally weak to moderately active assimilation; but after six to eight hours' exposure weak or none, assimilation in some cases not returning, but in others in one day being normal again.

The experiments with *Dendrobium* show that localized exposure produces a purely localized effect. With the stoppage of assimilation a change of colouration is generally associated, but is not an essential accompaniment thereto, for the power of assimilation commonly returns before any perceptible regeneration of the original intensity or shade of colouration takes place. The results obtained with *Pisonia alba* indicate that the effect produced is a photo-chemical one, and is not primarily due to, though it may undoubtedly be aided by, the heating effect of the sun's rays, the latter being the case whenever the leaf becomes at all wilted. It very commonly happens that when a leaf is exposed, the shaded side may retain, especially if the leaf is a thick one, a distinct power of assimilation, even though the palisade-layers composing the upper exposed surface are almost fatally affected. If a bifacial leaf is exposed, under surface upwards, it is much more likely to be fatally affected than when in the normal position.

Thick fleshy leaves do not wilt when exposed for prolonged periods; but, owing to the relatively slight amount of transpiration of which they are capable, they are very liable to be overheated by the absorption of the solar heat-rays. Thus in *Hoya fraterna* the insolation-temperature of the leaf is about 45° C., occasionally rising to over 50° C. The temperature was measured by suddenly bending the leaf around and pressing it against a delicate thermometer which was originally a little below the expected temperature. Since the general optimum temperature for assimilation probably lies between 25° C. and 35° C., steadily diminishing from 40° C. onwards, until at 50° C. or thereabouts the maximal temperature is reached [1], it follows that the leaves of such plants as *Hoya*, though probably their resistant powers in this respect are above the normal, have, when fully insolated, their assimilatory powers largely diminished by the high temperature which they attain, and may, when subjected to prolonged exposure, have their power of assimilation temporarily or permanently affected, partially or mainly from this cause alone. This is the case in the following experiments.

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[1] See Assimilatory Inhibition: Journal of Linnean Society, 1896, pp. 385 and 386.

Vanilla aromatica. After ten hours' exposure, leaves paler green; yield weaker extract of chlorophyll than normal; assimilation weak or absent in palisade-parenchyma, weak to nearly normal in spongy mesophyll. Next day assimilation active, except in few parts where faint or absent, the latter finally dying. Preparation difficult owing to presence of mucilage and raphides. Full insolation-temperature is about 40° C., occasionally rising to 45° C.

Hoya fraterna. Similar results to those with *Vanilla*, but the inhibition is more complete; the leaf is pale green to pale yellowish or brownish green, and in latter cases none or only a partial recovery takes place. Insolation-temperature 45° C. occasionally rising to 50° C. Leaves in exposed situations soon become yellowish, and assimilation is weak or absent. If kept in weak diffuse daylight, provided the change of colouration was not too pronounced, they become green again in one to two weeks, but show active assimilation long before this.

A cut stem of *Vanilla*, 4 ft. long, left entirely without water, remained living for a long period of time, the power of assimilation in the leaves being after one week fairly active, after two faint, and after three absent. But in the last case, on placing such a leaf with the cut surface in water, after two days a weak power of assimilation was shown. The stem and leaves began to wilt basally upwards after six weeks; after the seventh week a small leaf and root produced at the apex after separation from the parent plant ceased to grow; the leaves were thin and shrunken, the cells in the basal leaves dead and unplasmolysable, though the colour was but little altered. After ten weeks all the leaves are dead. *Vanilla* is therefore very resistant to drought, and any effects produced by insolation are not due to any excessive loss of water, but to the heating and photo-chemical effect of the sun's rays.

In the young green leaves of *Hoya fraterna*, when ¼ to ⅓ full size, minute starch-grains are present in the chlorophyll-corpuscles, and later, several larger ones are present in each; but it is only after the leaf is adult, and has been actively assimilating for some time, that the characteristic large oil-drops (soluble in ether and turning brown with osmic acid) appear in the assimilating cells. If such leaves are kept in darkness, in five days the oil-drops disappear in almost all the cells, but the starch is not perceptibly, or only very slightly, diminished in amount, whilst in ten to fifteen days almost every trace of starch has also disappeared. The protoplasm hence first absorbs and uses up the stored oil, and later the chlorophyll-grains yield up to it their stored carbohydrate. The slower disappearance of the starch is not due to the chlorophyll-grains being sick, for on examination after fifteen days in darkness a fairly active power of assimilation is shown. In *Dicranum scoparium*, *Musa*, *Vaucheria*, &c., the assimilatory products are at once converted into oil, starch-grains appearing in the chloroplastids only when a superabundance of assimilated material is formed. In *Hoya*, on the other hand, oil is formed only when an excess of assimilated material is present; it is the last to appear and the first to disappear. This peculiarity enables an interesting corroboration to be made of the results already obtained with *Hoya*. Thus in leaves, in which, without any fatal injury being inflicted, the power of assimilation is inhibited or reduced to a minimum by continued over-exposure, the oil originally present may entirely or almost entirely disappear.

In a previous paper[1] it has been shown that any agency which, when applied in intense or concentrated form, directly inhibits or prevents assimilation from taking place, may, when the application thereof is much weaker but prolonged, gradually induce in the chloroplastid a condition characterized by a temporary inability to assimilate. Thus when chloroplastids are subjected for prolonged periods to illumination by sunlight of natural intensity, though at first they continue to assimilate normally, their power of performing CO_2-assimilation is finally more or less markedly inhibited. Pringsheim[1] has shown that chloroplastids when exposed to intense and concentrated sunlight are killed and bleached only when oxygen is present. When subjected to such exposure in an atmosphere formed by an assimilatory mixture of hydrogen and CO_2 the chloroplastids remain green. It follows, therefore, that when exposed to concentrated sunlight, no assimilation of CO_2 or evolution of oxygen takes place. Hence there appears to be a maximal intensity of illumination at which the synthetic assimilatory processes are inhibited and cease, being finally replaced if a supply of oxygen be present by katabolic oxydatory changes. This appears to be in analogy with the general law, exemplified more especially in the effects of temperature upon the combustion of gases, that combinations between substances taking place at a relatively low temperature tend to be less and less complete as the temperature increases, and finally may be replaced by dissociatory changes taking place in the reverse direction.

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[1] Loc. cit., Journal of Linnean Society, 1896, pp. 364, 461.

PROTECTIVE MOVEMENTS BY THE LEAVES OR LEAFLETS.

Of the various modes by means of which tropical plants protect themselves against the injurious effects of over-exposure to sunlight, perhaps the most interesting and commonest is by means of active or passive movements of the leaves. It may be stated as a general rule, to which apparently there are no exceptions, that no tropical plant places or allows its leaves to be in such a position that the upper surfaces are at right angles to the sun's incident rays when at the zenith. In most exposed tropical plants, in which the leaves or leaf-stalks are not stiff and rigid, the leaves at midday assume a more or less drooping position when exposed to full insolation, this being due in the majority of cases not to any active movement of the leaf or leaf-stalk, but to a general diminution of turgidity, the somewhat flaccid leaf-stalk or leaf-base allowing the leaf in virtue of its own weight to assume a more or less pendent position. In a large number of cases, especially in the Leguminosae, the movements are not passive, but are active, the leaves or leaflets being para-heliotropic; i.e. when exposed to intense illumination place themselves parallel to the incident ray.

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[1] [illegible] pp. 326, 344.

This phenomenon has already been described by a number of observers, but many details are still unsolved. Thus the relative irritability of the different parts, the intensity of light forming a minimal and an optimal stimulus for movement, the relative efficiency of light of different colours, whether the position assumed bears any relation to the direction of the incident ray, and whether it is the leaf which perceives the stimulus and transmits it to the motile pulvinus, or whether the latter only is sensitive, being both the motile and the percipient organ, are all points requiring an experimental answer.

Mimosa pudica perhaps affords the most favourable subject for the experimental study of these questions, owing to its marked irritability and sensitiveness, and to its being one of the commonest weeds in both Buitenzorg and Peradeniya. A curious and interesting fact at once brought to light is that the pulvini of the primary and secondary leaves are affected differently to the pulvini of the leaflets. Thus the leaf of *M. pudica* reacts as a whole to the directive influence of sunlight, the movements of the pulvini being such as to place the leaf as nearly as possible at right-angles to the incident rays, if the illumination is sufficiently strong. In diffuse daylight the entire leaf-system is horizontally expanded, but in the morning and late afternoon, when exposed to obliquely falling sunlight, the leaf-system sets itself as far as possible at right-angles to it, the main petioles and secondary axes twisting or bending so that their upper surfaces face the sun. The leaves pointing towards the sun remain nearly horizontal or bend slightly downwards; those pointing away from the sun rise upwards, and may become nearly vertical if the sun be low down but strong and unclouded. The axes of the leaves to right and left remain horizontal, but twist so as to face the sun, chiefly at the secondary petiolar and partly at the main pulvini. The pulvini of leaves in an intermediate position partly twist and partly bend in setting the leaf axes in their proper position.

The pulvini of the leaflets respond in a very different manner. When exposed to strong and direct illumination, they cause the leaflets to rise up and fold more or less completely together, thus setting the surfaces of the latter parallel to the incident ray; when the light is weak or diffuse they cause the leaflets to expand horizontally. The pulvini of the leaflets appear to be somewhat more irritable at from 8 to 12 a.m. than they are later in the day. The leaflets commence to rise up a second or two after the sun falls upon them, and in a half to one minute under favourable conditions have risen up their full amount. Owing perhaps to the slightly lessened mechanical moment, the secondary petioles may also rise up slightly, but this is soon readjusted. If the leaflets are shaded after short exposure, they become fully re-expanded in from one to three minutes. Occasionally the reactions are nearly twice as rapid as the above, whilst after prolonged exposure the re-expansion may take place more slowly. If the stimulus is removed before the reaction is complete, the response continues for a short time in the original direction, both a latent period and an after-effect being clearly shown. A series of stimuli of short duration may, owing to the existence of a latent period, be summated and produce a response. Thus, successively exposing to the sun for two seconds and then shading for two seconds finally causes the leaf to rise up nearly to the full amount, though it takes a longer time for the movement to be completed. If alternately for two seconds in sunlight and four seconds in shade, the leaflet rises up, though only through about 15° to 20°. If alternately for one second in sunlight and ten seconds in shade, the leaflets remain fully expanded.

The angle which the leaflet assumes is nicely graduated to the intensity of the light to which it is exposed. Thus in only moderately strong sunlight the leaves rise up through an angle of from 30° to 50°, in weak sunlight from 15° to 30°; whilst if the sun is so shaded by clouds that it can barely cast a perceptible shadow, the leaflets rise up a few degrees only.

When the sun's rays fall somewhat obliquely, but not sufficiently so to cause the plane of the entire leaf to readjust itself at right angles to the direction of the incident ray, in the leaves the axes of which cross the sun's rays to right or left, the further row of leaflets appears to rise up more than the nearer one, so that in each pair of leaflets the angles made between the incident ray and the distal portion of each leaflet is the same, the leaflets apparently responding to the directive action of the incident ray. This appearance is, however, really due to the twisting of the secondary petiole setting its dorsal surface directly facing the sun, for as a matter of fact both sets of leaflets move through the same angle in relation to a perpendicular line falling between them on the petiolar axis to which they are attached.

In the quite young unopened leaflets naturally no sensitiveness to sunlight can possibly be perceived; but in young opened leaflets of about two-thirds the normal size the sensitiveness is even more marked than in adult leaflets, the former folding up when exposed to light of less intensity and expanding fully horizontally only when very completely shaded.

After prolonged exposure to unbroken sunlight for six to eight hours, the leaflets are all completely folded, and re-expand in the shade only very slowly. The petioles are generally in a more or less drooping position, the loss of water by transpiration in spite of the folded position of the leaflets hence having been excessive. The leaflets show a rather weak power of assimilation, which is in some cases barely perceptible, but becomes normal again over night.

If a layer of cold water is interposed between the sun and the exposed leaves, the leaflets rise up as usual but not quite so markedly (5° to 10° less), the difference being due probably to the slight loss by refraction and dispersion as the light passes from the air through the optically denser medium and out again. If sheets of coloured glass are interposed, in all cases the leaflets do not rise up so much as when exposed to direct sunlight; under blue glass the difference being least, under yellow more, and under red glass most marked. When exposed to intense sunlight under blue glass of not too great thickness, the leaflets may fold up nearly to their full extent; whilst when a double layer of thick red glass is interposed they may expand fully horizontally. Sachs and Bert have shown that in red or yellow rays from light of only moderate intensity the leaflets fold together, assuming the nyctitropic position, whereas in the blue rays from light of similar intensity they remain expanded. Hence when the blue rays are weak or absent the leaflets fold together, and when the rays are too intense they again fold together. It is obviously an advantage that the blue rays, when too intense, should cause the leaflets to fold together and hence be protected from excessive photochemical action; but that blue light of only moderate intensity should cause the leaflets to pass from the nyctitropic into the expanded position is only accidentally and indirectly an advantage, owing to the fact that under normal exposure the intensity of the blue rays is a sufficiently accurate measure for the plant of the intensity of the accompanying rays absorbed by the chlorophyll and used in assimilation. The leaflets respond not to the heat but to the light-rays of the sun, and of these rays it is the more refrangible 'chemical' rays which are most effective.

The following experiments show clearly that not only is the pulvinus the motile organ, but it is also the irritable perceptive organ and directly responds to the stimulus exerted by a change in the intensity of the illumination, the leaflet

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[illegible] VIII, 1870.

itself playing a perfectly passive part. If a well-defined strip of shade is thrown on the median portion of the secondary leaf, covering all the pulvini but leaving the leaflets exposed to full sunlight, in from two to four minutes the leaflets all become horizontally expanded. If now the leaflets are shaded but the pulvini exposed to sunlight, the leaflets assume the full sunlight position in from one to two minutes. If the leaflets, or the pulvini only, over a portion of a secondary leaf are shaded or exposed to sunlight, this part only responds. If the leaf is in the sunlight-position it is the basal part which should be shaded; but if the leaflets are fully expanded it is the terminal part of the secondary leaf which should be exposed to sunlight, as otherwise the overlapping non-moving leaflets interfere with the movement of the irritated portion and prevent the formation of the sharp line of demarcation between the shaded expanded and exposed folded portions which would otherwise be produced.

When the pulvini alone are shaded, it takes from a half to one minute longer for the leaflets to become fully expanded than it does when the entire leaf is shaded. If the pulvini of the basal pair of leaflets be shaded, the leaflets expand horizontally and remain so even though the whole of the rest of this pair of leaflets and of the entire leaf is exposed to full sunlight. Similarly if one pulvinus only is shaded, that leaflet only to which the shaded pulvinus belongs becomes or remains expanded. The same is the case with a single leaflet or pair of leaflets in all parts of the leaf, but they become or remain expanded only as far as the overlapping leaflets will allow.

If the sunlight be intense and the shadow thrown on the pulvini narrow, the amount of reflected light that reaches the pulvini may be sufficient to cause them to retain a slight angle (5° to 10°) with the horizontal. The interposition of a strip of red or yellow glass between the pulvini and the sun, causes them to expand nearly fully horizontally, whereas if a strip of blue glass is interposed, they remain nearly completely folded together. If the leaflets are completely shaded from above, but some illuminated from beneath by sunlight reflected from flat mirrors, the leaflets, the pulvini of which are illuminated, rise up, the others remaining horizontally expanded. If the light is somewhat concentrated by means of a plano-concave mirror, the leaflets re-act nearly as actively and rise up nearly as much as when exposed to direct sunlight falling from above.

Light therefore exercises no directive influence upon the pulvinus of the leaflet; it is only a change in the intensity of the illumination which acts as a stimulus, the movement always taking place in the same direction no matter how the light falls upon the pulvinus, and the angle through which the leaflets move being dependent upon the intensity of the stimulus.

As is well known, when the intensity of the light to which the plant is exposed falls below a given intensity, the leaf assumes the nyctitropic position; the primary and secondary petioles drooping whilst the leaflets rise up and fold together. When the evenings are bright and cloudless, the leaves begin to close in the tropics after 5 p.m., and by 5.30 p.m. are quite closed, a rough calculation showing that they thereby lose about the equivalent of five to ten minutes' bright midday illumination for assimilating purposes, which is a trifling and perhaps negligible amount. The folding together of the leaflets, along with the diminished temperature and closure of the stomata, besides hindering or preventing assimilation, also interposes a marked check upon transpiration, causing the plant, and more especially the pulvinus, to be temporarily in an even more highly turgid condition than is normally the case. If, when the full nyctitropic position has been assumed, the main pulvinus of the leaf is stimulated by touching the sensitive hairs on its under surface, it gives a marked response, and the turgidity of the upper surface of the pulvinus may be so much greater than that of the stimulated and originally convex surface that the leaf is bent beneath or across the stem, thus reversing the normal position of dorsal and ventral surfaces, and is supported against the action of gravity in this position for some time. The same stimulation applied to the expanded leaf at midday causes it merely to fall and droop downwards, the fall being largely a passive one, due to the action of gravity being no longer counteracted by the greater turgidity of the convex under-surface of the pulvinus. It must not however be concluded that the sleep-position assumed in the evening is simply due to the general increase of turgidity consequent upon the diminished transpiration. The folding up of the leaflets, which is one of the main factors causing the diminished transpiration, is directly due to the stimulating action of the fading light; for any general increase in turgidity tending to make that of the upper surface more nearly equal to that of the under surface of the pulvinus would tend, not to close the leaflet, but to keep it expanded, were it not overpowered by a more powerful direct stimulus. Besides, were the latter not the case, we should expect to find the leaflets expanding during the night as the normal relation between transpiration and absorption became restored; whereas, as is well known, they remain folded together until caused to expand by the early morning light. The fall of the main and secondary petioles may, however, partly be brought out in the manner above described, for they rise somewhat during the night; but even here the movement is mainly due to the direct stimulating action of the absence of light, for it is only on re-exposure to light of adequate intensity that the normal fully-expanded condition is assumed.

Equally exposed plants of *Mimosa* growing in the one case in dry sandy and in the other in damp humid soil, close at the same time in the evening, and not earlier in the latter case than in the former. If a portion of a plot of plants be kept dry and the rest well watered, with equal exposure, and the plants being to commence with fully expanded, the leaves and the plants in the latter may close five to ten minutes earlier than in the former; but this simply shows that the more turgid plants respond somewhat more rapidly to the stimulus due to the fading intensity of light than do the less turgid ones.

In order to keep the leaflets of *M. pudica* fully expanded, exposure to light of a certain tonic intensity is necessary; exposure to light of greater intensity than this causing a paratonic closure, whilst too feeble illumination causes an atonic closure.

A number of other Leguminosae were examined with regard to the paraheliotropic properties of their leaflets, and though they vary much in degree of irritability and the movement of response is not always of the same kind, nevertheless the general results, especially with regard to the pulvinus being the organ which not only responds to but also perceives the stimulus, were essentially similar to those given by *M. pudica*.

An unnamed *Acacia* (165[1]) is nearly as sensitive as *M. pudica* to both contact and exposure to sunlight. Thus the leaflets when lightly tapped rise up and fold together. If exposed to full sunlight they fold completely together in two to three minutes, expanding fully again in five to eight minutes when well shaded. If the pulvini alone are shaded, the leaflets expand nearly completely horizontally, and the movement is but little slower than it is in *Mimosa* under similar conditions. In sensitiveness to contact and to insolation, the leaflets of *Robinia Pseud-acacia* are almost identical with those of the preceding plant. In another unnamed *Acacia* (169[1]) the leaflets are not quite so sensitive, a smart rap causing a partial folding, whilst when exposed to full sunlight they are not quite so closely approximated together and when shaded re-expand more slowly.

Dalbergia zeylanica, Roxb., though not sensitive to mechanical irritation, is very nearly as sensitive to, and shows nearly as rapid a response to, exposure to full sunlight, as does *Robinia*.

In *Caesalpinia*, *Dalbergia*, and several Acacias, if the leaflets are exposed to full sunlight but the pulvini are shaded, the former become markedly expanded in a quarter of an hour, and in half an hour are nearly horizontal. If fully exposed to sunlight, the leaflets rise up or twist to their full extent in ten to twenty minutes. The leaflets of several species of *Caesalpinia*, of *Dalbergia linga*, and of *Desmodium velutinum*, D. C., twist, so that the longitudinal axis is at right angles to the lamina or parallel to the sun's rays, the proximal edge of the leaflet being uppermost. At the same time the leaflets may in some cases rise somewhat, but the main and secondary petioles generally droop more or less after prolonged exposure. In this case also the movement of the leaflets is always the same no matter what the angle at which the incident rays fall, the intensity of the light alone acting as a stimulus to movement. Likewise, if the pulvini alone are shaded, the flat surfaces of the leaflets become horizontal, the dorsal (morphologically ventral) surface being directed upwards; but if at the same time the leaf be powerfully illuminated from the side or from beneath, the leaflets, though shaded from the sunlight falling from above, retain their paraheliotropic position.

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[1] The numbers are the Buitenzorg Garden numbers given to enable future identification to be made, specific identification being impossible at the time owing to the lack of flowers and fruit.

In *Albizza sp.?* when exposed to sunlight the leaflets fold forwards and also twist so that the proximal edge is uppermost and the lamina vertical. If the pulvini are shaded, the leaflets begin to expand and twist in five minutes and attain nearly to their normal shade position in fifteen minutes. *Albizza saponaria*, Bl., and *Calliandra haematocephala*, Hsskl., are similar in all respects, with the exception that the movement is slower and takes nearly twice as long before it is completed.

The leaflets of *Cassia montana*, Heyne, when exposed to sunlight fold together forwards, more or less, whilst the pulvinus twists so that the leaflets droop downwards with the under ventral surface directed outwards and in some cases slightly upwards. If the pulvini are shaded, the leaflets begin in ten minutes to expand, and rise up and twist so that the dorsal surfaces face upwards, a nearly normal shade-position being assumed after twenty to thirty minutes. The proximal surface of the pulvinus, which is uppermost in the sunlight-position, has a moderate amount of red dye in the epidermal cells covering it. Stomata are abundant on the under surface of the leaf, but none are present on the upper. Hence the movement is evidently a means of protection against light alone and not against excessive transpiration; for if the latter were the case we should expect to find the ventral stomatic surfaces being apposed to one another. The outer surfaces of the epidermal cells of the leaflet are curved in outline, the curvature on the upper surface being more marked and conical, on the under flatter and more rounded. This would probably indicate according to Stahl[1] that *Cassia montana* is specially adapted to suit a shady habitat. The sunlight position which the leaflets assume, though sufficient as a protection against not too prolonged exposure, is, owing to the way in which the ventral surfaces are exposed, not so adequate a protection as in the other cases already described.

The bilobed leaf of *Bauhinia,* though slow to react to sunlight, forms nevertheless, owing to the large size of the pair of leaflets of which it is composed, to the manner in which these are hinged together in the median line for a great part of their length, and to the pulvini being partially bifid, interesting material for experimental study. In *B. elongata* the leaflets, when exposed to full sunlight, fold almost completely together, each rising up through an angle of 80° or more. If the pulvinus at the base of the pair of leaflets be shaded, the area shaded being about 1 sq. cm., whilst the area of the leaf left exposed is from 100 to 200 times greater, in a quarter of an hour the leaflets are seen to be expanding, in half an hour they make an angle of 15° to 20° only with the horizon, and in one hour they are nearly horizontal. On then re-exposing fully to sunlight, or on exposing the pulvinus alone, the leaflets begin to rise in five minutes; in ten minutes they have nearly, and in fifteen fully, assumed their normal position. A leaf exposed for eight hours to continuous sunlight showed, if the leaflets were allowed to fold together, a fairly active power of assimilation; but another in which, by shading the pulvinus, the leaflets were caused to remain nearly horizontal, showed after the same time only a moderately active power of assimilation, and in parts faint or none; the next day the remaining portions of the leaflets showed fairly active assimilation. If one half of the pulvinus be removed, the cut surface being smeared with vaseline, the leaflet of that side sinks and can no longer respond to a change in the illumination. The other leaflet, however, still responds, though not quite so markedly as normally.

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[1] Stahl, Ann. d. Jard. Bot. de Buitenzorg, Vol. xiii, 2e partie, 1896. 'Sammetblättern,' &c.

B. tomentosa, with a much larger leaf and pulvinus, responds more slowly than does *B. elongata*. When the pulvinus is shaded it takes from one to two hours for the two lobes to become nearly horizontal, and, when exposed to sunlight, from a quarter to a half hour to close up to the full extent possible. The leaflets can only close completely together when quite young; when older a slight angle (15° to 20°) always remains between the two leaflets, probably owing to a slight hardening of the pulvinar tissue and to the increased elasticity and incompressibility of the tissue forming the longitudinal hinge between the two lobes. In both *B. elongata* and *B. tomentosa* the pulvini are covered with brown hairs, and both the epidermal and outer cortical layers are dark brown in colour. This is less marked when the leaf is young, and hence probably arises the fact that the quite young leaves fold together when exposed to light of only moderate intensity. In the older, more resistant, stouter, and less transparent leaf, the sensitive pulvinar tissues are largely shielded from the penetration of light, and respond, causing the leaflets to fold together, only when the light is of considerable intensity and the exposure prolonged. The pulvini of the more sensitive leaflets of *Acacia*, *Albizza*, *Dalbergia*, and *Caesalpinia*, are all glaucous and green, and are exposed to the sun's rays in almost every position which the leaflets may assume.

In *Bauhinia*, when the illumination is at all oblique, the folding of the leaf-lobes may cause the pulvini to be shaded and any further movement to cease.

Both *B. elongata* and *B. tomentosa* have an abundance of stomata present on the markedly hairy under epidermis of the leaf, the upper surface being slightly hairy and possessing a few scattered stomata. Hence in the folded position the rate of transpiration can only be very slightly diminished. In *Dalbergia zeylanica, D. linga, Albizza saponaria, Calliandra haematocephala*, and *Caesalpinia*, stomata are present on the under surfaces of the leaflets only, the upper epidermis having a slightly or markedly thicker cuticle than the under, and being without stomata. In *Albizza saponaria, Calliandra haematocephala*, and *Dalbergia linga*, it follows from the position the leaflets assume, that no checking but rather an increase of transpiration is likely to result when the leaves are exposed to sunlight. In *Dalbergia zeylanica*, the leaflets of which fold together in pairs, even though no stomata are present on the upper surfaces, the folded position of the leaflets will interpose a slight check on transpiration; whilst in *Mimosa* and *Acacia*, owing to the presence of stomata on the upper surfaces and to the overlapping of the leaflets when folded, the check must be very marked. In *Acacia* (165) and in *Robinia Pseudacacia*, stomata are abundant on the under surface and on the outer half of the upper, becoming fewer towards the base of the upper surface of the leaflet, whilst at the base none are present. Hence in these two plants stomata are absent from that part in which, when folded together, there is least chance of any transpiration taking place.

The paraheliotropic position is very evidently assumed solely in order to protect the chlorophyllous layers of the leaflet from exposure to too intense illumination, though it may, as a secondary, and perhaps accidental, effect in a few cases help to guard against excessive transpiration.

PROTECTIVE RED DYE.

In a very large number of cases, and more markedly in the tropics, the leaf, especially when young, is partly shielded from the effects of exposure to light by the presence of a protective red dye. Stahl, in a recent publication[1], has expressed certain theories with regard to the function of erythrophyll in plants, which cannot be passed over without criticism. In opposition to the generally accepted view, Stahl holds that the importance of the red dye is that it acts, not as a protection against light at all, but first, as a warning or frightening (*abschreckende*) colouration, and secondly, and most importantly, as a heat-absorbing medium.

As regards the importance of the red dye as a frightening colouration, Stahl himself shows that it only applies to higher animals in which the powers of vision, and more especially of colour-perception, are well developed; and even then the deterrent effect is by no means marked, and ceases to become evident when the animal is hungry. Obviously, with animals such as snails, the main depredations of which are carried on in darkness or in partial obscurity, when the red colouration cannot be distinguished, it can hardly exercise any deterrent effect in virtue of its colour. It may safely be concluded that any value of the red dye as an '*abschreckende*' colouration is quite accidental and secondary, though it may in some cases be of undoubted use to the plant. A similar instance, in which a special peculiarity developed for a particular purpose has acquired accidentally a minor secondary importance, is afforded by *Mimosa pudica*. In this plant the folding up of the sensitive leaflets, which takes place when the plant is agitated by the wind, is undoubtedly of primary importance as a protection against mechanical injury or drought, to both of which the plant is very sensitive and liable. When a browsing animal, such as a goat, approaches a clump of

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[1] Stahl, Ueber bunte Laubblätter: Ann. d. Jard. Bot. de Buitenzorg, Vol. xiii, 2ᵉ partie, p. 137, 1896.

M. pudica, and agitates any part of it at all strongly, the green appearance disappears at once, and only an apparently withered clump, in which the hard and prickly stems are most conspicuous, remains; the consequence being that the animal either turns away or passes through the clump of *Mimosa* to reach more congenial and less bewildering pasturage. In a country with many browsing animals this peculiarity cannot fail to be of considerable, though only accidental, importance.

As regards the second part of Stahl's theory, there is no doubt whatever that the presence of erythrophyll in the cell-sap will cause a leaf to absorb more light, and also more heat, than it would otherwise have done; but the question is whether the heat-absorbing property of the red dye is not merely secondary, and, perhaps, in many cases, a disadvantage to the plant. In the case of alpine plants, Kerner von Marilaun[1] had already pointed out that the heat-absorbing power of the red dye may be of accessory importance to its light-protective function.

Engelmann[2] has shown that the red pigment erythrophyll allows, in a typical case, about 90 per cent. of the orange rays which are most useful for assimilation, 10 to 30 per cent. of the green and yellow which are least useful, 50 per cent. of the blue which are somewhat more useful, and 80 per cent. of the violet, rays to pass through.

Pringsheim[3] found that when exposed to concentrated blue or green sunlight in the presence of oxygen, the chlorophyll-grains over an exposed area of an end-cell of *Chara* are killed and bleached in 5 minutes; whereas when exposed to the concentrated red rays and kept cool, they remain living for a longer time, only beginning to bleach after 20 minutes' exposure. The red dye therefore, whilst allowing as much as possible of the light useful for assimilation to pass through, absorbs as much as possible of the rays exerting the greatest photo-chemical effect upon the chlorophyll and the protoplasm. Were the primary and most important function of the red pigment to act as a heat-absorber, it would be natural to expect that it should show a marked absorption of the dark heat-rays which do not appear to be used in chlorophyllous assimilation, instead of its absorbing for the most part the green and yellow rays, in which the heating effect is comparatively slight. Engelmann (l. c.) does not, however, ascribe any power of absorbing the dark heat-rays to erythrophyll. Stahl (l. c.), it is true, finds that red leaves, when exposed to dark heat-rays, show a rise of temperature of from 1° to 2° over similar green leaves similarly exposed. The explanation of this probably lies, however, in another observation of Stahl's, namely, that red leaves have fewer stomata than the green ones, the consequently lessened transpiration in the red leaf being probably the cause of the difference in temperature. It is extremely probable that in the water distributed throughout its substance, the leaf possesses as sufficiently powerful an absorbent of the dark heat-rays as is needful. The very fact that in spotted leaves the red areas contain fewer stomata than do the neighbouring green ones, is very strongly in favour of the view that the slight additional absorption of heat and consequent acceleration of transpiration is a disadvantage which needs to be guarded against, and which is in this manner adequately compensated for. As Stahl shows, green leaves or green areas of the leaf, when exposed to *sunlight* in *dry* air, give off more water-vapour than red leaves of the same plant or red areas of the same leaf do.

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[1] Pflanzenleben, Bd. i, p. 364.

[2] Engelmann, Bot. Zeit., June, 1887.

[3] Pringsheim, Pringsh. Jahrb., Bd. xii, 1881, pp. 326-344.

Kerner von Marilaun[1] mentions that *Satureja hortensis*, grown fully exposed to sunlight in his alpine garden, develops an even greater abundance of the protective red dye and flourishes, whilst *Linum usitatissimum* remains green, grows badly, and dies before flowering. From this he concludes that the red pigment is important as a protection against intense illumination. Stahl (l. c.) finds that, in the

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[1] Pflanzenleben, Bd. i, p. 364.

Alps on clear nights, *Linum* is unable to empty its leaves of starch, whilst *Satureja* does so, and hence concludes that this is due to the red leaves being warmer during the night, an assumption which is not justifiable without direct experimental evidence, for it has yet to be proved that the red pigment has any special power of absorbing dark heat-rays: moreover, in correlation with the fact that the red leaf absorbs heat in slightly greater amount than a green one does, it would be natural to expect that the former would also radiate heat more rapidly than the latter when the temperature falls during the night. The above observations cannot be said to point one way or the other. All they show is that *Satureja* is more adaptable to an Alpine habitat, and with this adaptability the presence and increased formation of the red dye may be a more or less important correlative factor; whilst *Linum*, under such conditions, is unhealthy, and amongst other signs of sickness a weakening of the translocatory powers may be one symptom. Costerus[1] has, however, shown that, in most tropical plants, but little starch is removed during the night; so that in the tropics, at any rate, the retention of an abundance of starch in the leaves over night is a normal phenomenon, and does not indicate that the plant is in an unhealthy condition.

Stahl supposes that in the tropics the heat-absorbing function of the red pigment is to increase the amount of transpiration, and in the plants of temperate regions to raise the temperature of and hence give rise to more active metabolism in, more especially, the young growing organs. He points out that in the tropics many plants affecting shady habitats have a marked and deep red colouration, and that in many cases the red dye is present only in the under epidermis or spongy mesophyll. The explanation of the presence of a marked red colouration in the leaves of many shade-plants is readily explained as being necessary to guard against the partial or complete assimilatory inhibition to which such shade-plants are especially liable when accidentally exposed to prolonged intense illumination.

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[1] Costerus, Ann. d. Jard. Bot. de Buitenzorg, p. 73, Vol. xii, Pt. I, 1894.

In trees or shrubs growing more or less in the open and with foliage which is red when young, it can plainly be seen that the young foliage formed in more shaded positions is less red than that which is more exposed; whereas according to Stahl's theory the reverse should be the case. The more exposed leaves also retain their red colour longer and to a later stage of development than the shaded ones, in those cases in which the leaves lose their red colour when adult, as is shown well by *Ancistrocladus Vahlii.* Owing to the tonic stimulating action which light exercises, the more exposed leaves are larger, broader, and develop more rapidly, than very much shaded ones. Hence, owing to the chlorophyllous tissues developing and reaching their adult and most resistant condition more rapidly, the red colouration may disappear from the more exposed leaf in an actually slightly shorter time, though it persists to a later stage of development and size in the more exposed leaf than in the markedly shaded one.

That many plants growing in the shade form a greater or less amount of red pigment is quite true: but in all the cases observed the red colour is most marked when the plant is growing in more exposed situations, and in extremely shady habitats it may almost or entirely disappear. The inference simply is that such plants are extremely sensitive to and readily injured by exposure to light of at all marked intensity: whilst it has already been clearly established that very marked differences do actually exist in the sensitiveness of different plants to light in correlation with the amount of exposure to which they are subjected in their natural habitat.

In the Filicineae it is possible to formulate the general rule that hygrophilous Ferns growing in damp and shady places are always green; whilst in more or less xerophilous Ferns, growing in more exposed and drier habitats, a marked red colouration is frequently present. Thus in *Blechnum orientale*, growing in an exposed and rather dry situation in the gardens at Peradeniya, the young leaves have a marked red colouration, and are at the same time protected from excessive transpiration by the presence of numerous stalked mucous glands of epidermal origin. The red colouration is most marked when the leaf is nearly fully expanded and about two-thirds its full adult size, being less marked in the young inrolled leaflets than in the still young but just expanded ones. As the leaf becomes adult, the red colour rapidly diminishes from the base upwards and the mucous glands wither away and disappear. The red dye is formed in the mesophyll-layers only, and chiefly and most markedly in the cells of the palisade-parenchyma. As the dye disappears the special protection which the mucous glands afford against excessive transpiration is no longer needed, and the glands dry up.

As regards the cases in which the red dye is present only in the under epidermis or in the spongy mesophyll, it must be remembered that in many such cases the young leaves expose their ventral surfaces to the light. Thus in *Musa* the young leaves are vertical and rolled up with the ventral (under) surface outermost; and in one variety of *Musa* a red dye develops on the under exposed surface, disappearing as the leaf unrolls and expands horizontally. The adult leaves of *Musa* (see above, p. 440) are quite resistant to even prolonged insolation, but the exposed portions of the young leaves are more sensitive, and may have their power of assimilation weakened or temporarily inhibited by a full day's insolation.

In *Uncaria sclerophylla* the young leaves have a red dye in the under epidermis only, which is also covered by adpressed sclerotic hairs. The young leaves are at first folded longitudinally, with the under (ventral) surfaces outwards; and commonly even as they expand and before the fixed light position is taken up, the ventral surface is more exposed than the dorsal. The red dye acts as a protection against sunlight, whilst the adpressed hairs guard against excessive transpiration. The dye begins to disappear when the leaf is from one-third to one-half its full adult size, and is almost entirely gone when two-thirds grown. Young expanded leaves (5 cm. long by 2·2 cm. broad) show normally a weak but distinct power of assimilation, but after eight hours exposure to full sunlight, with the dorsal surface upwards, assimilation is faint or absent, some leaves being fatally injured; whilst if the ventral surface, exposure of which in an adult leaf of a shade-plant sensitive to exposure produces more effect than does exposure of the dorsal surface, is subjected to insolation for the same time, a weak or faint power of assimilation is shown, and the leaves all recover and show next day a normal weak power of assimilation. In a fully adult green leaf (13·5 cm. long by 6·5 broad) after eight hours' exposure, a moderately to fairly active power of assimilation was shown.

An unnamed epiphytic Aroid, growing in the Canarien Allée near the main entrance of the Buitenzorg Gardens, has the young leaves rolled up vertically with the ventral (under) surface outwards, a red dye being present in the spongy mesophyll-cells and also around the vascular bundles. As the leaf expands horizontally, dorsal surface upwards, the red dye lessens in amount; but in adult leaves a slight amount is still present. In other plants the red dye might remain in this position in the adult leaf as a vestigial survival of that present in the young leaf, and without necessarily having any functional importance.

In many Scitamineae it is the under surface of the leaf which is red. *Alpinia officinarum*, Hance, has a purplish-red colour, on the under surfaces only, of the nearly vertically erect leaves. From the very young leaves the red colour is absent; it appears as soon as the leaf is exposed, and is most marked when from one-third to one-half full grown; after this it decreases in amount, disappearing at the edges and sides first, and finally in the fully adult leaf being totally absent. *Belemcanda chinensis* is exactly similar with regard to the distribution and appearance of the red dye, but the dye does not commence to disappear until after the leaf is fully adult and the young sensitive growing tissues no longer need that protection from light which, owing to the marked exposure, when young, of the ventral surface to illumination, was peculiarly necessary. In various Zingibers, Marantas, &c., in which the under surface of the leaf is red, the leaves when adult are horizontal with the ventral surface downwards, and the red pigment disappears in the post-adult stages. In some cases the leaves remain partly erect, in which case a slight red colouration may persist on the under surface.

When exposed to strong illumination, *Mimosa pudica* acquires a distinct reddish colouration on the upper exposed surfaces of the petioles and younger branches, which is slight or absent in the young still folded leaflets; in the adult leaves the red colouration is especially marked, but is restricted to the under surfaces of the leaflets. In shaded plants the red colouration is but slight or absent, and this is commonly the case in plants grown in European hot-houses. The red dye is formed in the epidermal cells at the edges of the leaflets and in those parts of the under surface which are exposed when the overlapping leaflets are all folded together. At the apex of the leaflet the under epidermis has a thicker outer wall and cuticle than the upper epidermis, but in the basal part of the leaflet this difference does not exist; if anything, the upper epidermis and cuticle are here slightly thicker than the under. On the basal pair of leaflets the under epidermis and cuticle, which are outermost and entirely exposed when the leaflets are folded, are thicker than the upper all over the leaflet. On the upper surfaces of the leaflets, stomata are abundant and increase in number towards the apex, being especially abundant at the extreme apex. The average number of stomata on the under surface is slightly greater than on the upper, but not quite so abundant as at the apex on the upper surface. On the under surface at the apical one eighth to one-twelfth of the length of the leaf, i.e. over the portion which, being exposed when the leaflets fold together, has a protective red pigment, no stomata at all are present. Of the basal pair of leaflets belonging to one of the outer petioles the outer one is generally larger and better developed besides being more exposed than the inner and in it the adaptive modifications are most markedly developed. The entire under surface is markedly red and hairy. The outer wall and cuticle of the epidermal layer, all over the under surface of the leaflet, are thicker than on the upper surface. Stomata are present all over the under surface, but only one-third to one-fifth, or less, of the number present on the upper surface, and the guard-cells have markedly thickened and cuticularized walls. On the upper surface stomata are abundant all over the leaflet, being less abundant at the base and especially abundant at the apex.

When therefore the leaflets of *Mimosa* are folded together, only the red and hairy parts of the under surfaces of the overlapping leaflets are exposed, whilst the fully exposed under surfaces of the basal pair, and the exposed edges of the other leaflets, are markedly red and hairy and contain as compared with the upper surfaces but few or no stomata. The red dye protects the exposed portions of the more sensitive under surface from any excessive photo-chemical action caused by the unnatural exposure, whilst at the same time transpiration is reduced to a minimum.

The leaf of *Mimosa* is very thin, consisting besides the upper and under epidermis, of a single layer of palisade-parenchyma with large and numerous chlorophyll-grains, and of two or three layers of spongy mesophyll. The plant is very sensitive to drought and is readily injured by it, the least scarcity of water causing the leaflets to fold and to remain folded for some time after the plant has been fully supplied with water again; whilst if the drought be at all prolonged the leaflets may never recover or re-expand. It is hence obviously an advantage that, whenever a wind of sufficient velocity to agitate the leaflets is blowing, any excessive and injurious transpiration which it might cause should be guarded against by the mechanical stimulation causing the leaflets to fold together. At night-time the folded position of the leaflets and the hairiness of the exposed portions act as a protection against the loss of heat by both transpiration and radiation.

On the upper surface of the pulvinus the epidermis bears some resemblance to a columnar epithelium, the outer ends of the cells being rounded or conical, whilst the cell-cavity is filled with a red sap. This layer probably acts as a protection to the sensitive pulvinar tissues, preventing them from being too readily affected by light falling from above. As has been already shown, the pulvinus is, probably owing to this fact and to the transparency of the pulvinar tissues, almost or quite as readily affected by illumination from beneath as from above; the only condition necessary being that the light when it reaches the sensitive pulvinar tissues shall be of sufficient intensity to act as a stimulus.

From the foregoing it is clear that the red colouration found on the parts of the under surfaces of the leaflets of *Mimosa* which are exposed when folded, comes under the same category as in the plants previously mentioned, being for the purpose of shielding the transparent spongy mesophyll, in which the higher processes of synthetic metabolism, as distinguished from the simpler primary assimilatory processes, are probably especially active, from the injurious effects of the exposure to which it might wholly or in part be liable when the leaflets are folded together.

In some cases, however, such as in certain Begonias, in which the leaf is horizontally expanded and the red dye is present only on the under surface in the adult leaf, Stahl's interpretation of the functional importance of the red colouration may be the correct one; for even excluding all such cases occurring in cultivated garden-plants in which the peculiarity might be due to artificial selection, still cases remain for which this explanation does not suffice. Granting that the red pigment must always be formed for a useful and definite purpose, its well-defined and apparently purposeful distribution precluding the idea of its being an accidentally coloured useless waste product of metabolism, it is difficult to see in such cases, even though they are but few in number, what other useful function it could possibly have.

As regards the young hanging-foliage of certain tropical trees, Keeble[1] has already shown that the hanging position acts as a protection against excessive transpiration which is very apt to injure the young leaves, and that when in addition a red dye is present the decomposition of the chlorophyll by exposure to strong light is thereby largely prevented. The temperature-determinations which Keeble made at the upper and under surfaces of red and green insolated leaves of *Amherstia nobilis*, seem to point to the following explanation. On the upper surface of the red leaf the temperature averages 1°C higher than on the upper surface of the older green leaf, owing to the transpiration being less and the absorption of heat being greater in the former case than in the latter; whilst at the under surface of the green leaf the thermometer averages a degree above that beneath the red leaf, owing to the green leaf being more transparent to radiant heat than the red one is. Any injurious increase in the rate of transpiration, which might be caused in the young red leaves by the slight increase in the power of heat-absorption which the red colouration gives them, is prevented by their overlapping and hanging position.

It is evidently of considerable interest to know exactly what power of assimilation the young leaf possesses when in the hanging position. The following observations made in the usual manner elucidate this point, the measurements in length (L) and breadth (B) being given in terms of those of the fully adult leaf:—

Saraca declinata, L $\frac{8}{17}$, B $\frac{6}{11}$. Very pale yellowish green, apical half pinkish white. No power of assimilation perceptible except along the veins, here the chlorophyll grains are small pale yellowish green and faint evolution of O is shown. L $\frac{4}{7}$, B $\frac{7}{11}$. Pale yellowish green, distinctly green along the veins, apical portion pinkish white. Chlorophyll-grains moderately large, weak assimilation, moderately active at base near ribs, in apical portion faint or imperceptible.

L $\frac{5}{7}$, B $\frac{8}{11}$. Chlorophyll-grains fairly large and green, assimilation active to moderately active, leaflets green and expanding.

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[1] Annals of Botany.

Brownea capitellata, L $\frac{1}{2}$, B $\frac{5}{13}$. Midrib pale green, leaf inrolled longitudinally, chlorophyll-grains quite small and pale yellowish colour, no assimilation perceptible. L $\frac{11}{16}$, B $\frac{8}{18}$. Leaf just unrolled, pale brownish green along veins, here in parts faint assimilation, in rest of leaf none and only faint greenish colouration. L $\frac{18}{16}$, B $\frac{10}{18}$. Expanded and nearly fully green, assimilation moderately to quite active.

Amherstia nobilis, Wall, L $\frac{5}{14}$, B $\frac{11}{15}$. Chlorophyll-grains small and pale yellowish green. No assimilation perceptible. L $\frac{1}{2}$, B $\frac{4}{7}$. Assimilation weak to imperceptible. L $\frac{19}{28}$, B $\frac{9}{15}$. Chlorophyll-grains fairly large, still somewhat yellowish green, assimilation weak. L $\frac{25}{28}$, B $\frac{14}{15}$. Still quite red but reddish colour disappearing at apex, and leaflets commencing to rise and expand. Chlorophyll-grains fairly large and yellowish to nearly normal green, assimilation weak to moderately active. Leaflet on cut branch in water exposed for five hours to full sunlight slightly fading in colour, assimilation imperceptible, being next day weak to moderately active. L $\frac{1}{1}$, B $\frac{1}{1}$. Adult fully green leaf, assimilation active. Leaflets on cut branches insolated for five hours show fairly active assimilation, but after a whole day's insolation, assimilation is fairly active, to weak or in some cases imperceptible, next morning being fairly active.

In *Amherstia* it is only when the red colour disappears and the leaf is expanded and green that assimilation becomes fully active. In the hanging position the assimilatory power of the leaflets being feeble or absent, the loss to the plant, by the non-exposure of the leaflets to light, of material which might possibly have been assimilated, is but trifling; whilst the gain, in the protection afforded against excessive transpiration or exposure to strong illumination and in the rapidity with which the young leaflets are thereby enabled to develop, is very great. If branches of *Amherstia* are bent so that the red hanging foliage-leaves are exposed to as much sunlight as possible, and the leaflets are supported on wires and spread out in a horizontal position, they may wilt or fade at the edges or tip or over irregular areas in the lamina, the growth of the leaflets is retarded and at the same time the exposure to excessive illumination causes a distinct retardation of the growth and turning green of the chlorophyll-grains. If the leaflets are merely spread out horizontally, but not otherwise exposed, so that the rate of transpiration is markedly increased, they may become wilted, and their development and rate of growth is slightly retarded. This is hence certainly a case in which any power of increasing the rate of transpiration which the red dye may possess in virtue of its feeble action as a heat-absorbing medium, does not seem to be of any advantage but rather a slight disadvantage to the plant, were it not guarded against by the hanging and overlapping position of the leaflets; whilst the protection which the red dye affords against the retarding action that light of too great intensity exercises on the growth and development of the young and especially sensitive chlorophyll-grains is clearly of the highest importance. The young leaf is not to be regarded as an assimilatory organ, the metabolism of which demands a large supply of mineral salts and hence also of water, but as a growing organ to which nourishment is supplied in soluble and concentrated form, and in which a comparatively small amount of transpiration suffices, along with the strong osmotic currents maintained by the continual removal of the supplied food-material, to provide it with all it requires.

If the primary function of the red dye in the tropics were to increase the amount of transpiration, then it would be only natural to expect that it would be formed in greatest abundance where the temperature is lower and the air more nearly saturated with water-vapour. The very opposite is however the case. Thus at the foot and sides of the volcanic mountain of Gedeh, and in the valleys around, very many plants have a reddish colour, especially in the young leaves. As one ascends this becomes less marked, until at Tjibodas and in the forests above it (4,500 ft. to 6,500 ft.), the number of plants showing a red colouration, and the intensity of the latter when present, reach a minimum. The vegetation at this elevation is almost entirely green, a few plants only, especially if growing in open clefts or glades in the forest, having more or less reddish young leaves. Yet it is just here where a power of stimulating transpiration is apparently most needed: for at this elevation the air is, during the greater part of the time, at or near saturation-point. On the other hand if the red pigment acts as a protection against sunlight, it is easy to understand why here, where the sun rarely shines for more than a few hours daily and then generally through a haze of clouds, the protective red pigment should almost entirely disappear; for it is just the more refrangible photochemical rays which the air saturated with water-vapour absorbs in greatest amount.

At the summit of Pangerango (10,000 ft.) and for a few hundred feet below, the plants are in one or more characteristics markedly xerophilous (hairs, wax, thick epidermis and cuticle, few stomata, mucilaginous or fleshy tissues, reduced leaf-surface, &c.), and very many have a pronounced red colouration either in the young leaves and shoots or in the adult leaves as well. Here, just above the cloud-belt, the exposure to light is greater both in amount and intensity; whilst the drier air, the lower atmospheric pressure, and the more prevalent wind, all render the rapid evaporation of water easy. From too intense exposure to light the plants protect themselves largely by means of the red pigment; whilst the xerophilous character which the plants assume guards them against excessive transpiration, to which the difficulty of absorbing water in sufficient amount when the ground temperature is low but the temperature around the aerial parts relatively high, a frequent occurrence at such elevations, renders them especially liable. It appears therefore that where transpiration is easy but the exposure to light great, the red colouration is most marked, but where transpiration is difficult and the exposure slight, it diminishes or disappears.

In Java at the commencement of the wet S.W. monsoon and in Ceylon at the rainy commencement of both monsoons, the vegetation acquires a more marked reddish tinge than in the dry periods between the monsoons. This is, however, simply due to the fact that the young foliage, which in most tropical plants is more or less tinged with red, is very much more abundantly formed at this period than during the dry season. Even during the wet season in West Java, there is almost always bright sunlight until mid-day, lasting often till 3 or 4 p.m., and occasionally all day; so that the young foliage which the rain has caused to be produced in such abundance is exposed for six hours on the average to very bright illumination, the sunlight from 9–12 being the brightest of the day. Hence the protective red colouration is perhaps quite as necessary during the wet season as during the dry.

An interesting fact noticed by Stahl (l. c.) is that in the adult leaf the guard-cells of the stomata are always without any red dye even though it may remain present in the epidermal cells of the upper surface. The importance of this Stahl concludes to be that the guard-cells remain cooler, transpire less, keep open longer, and hence promote transpiration and assimilation. However, Stahl also points out that in many plants, especially in spring, the epidermal cells immediately around the stomata have a red sap, and are thereby enabled to heat the points at which the water-vapour escapes and hence the guard-cells also.

Instead of adopting either of these contradictory explanations, it is perhaps simpler if we regard the absence of the protective red dye from the guard-cells of the stomata as being due to the fact that they are essentially organs which react to light: hence it is important that they should be exposed to the same intensity of light as is falling on the surface of the leaf in which they are present, even though a certain risk of permanent injury may thereby be incurred.

Wiesner[1] states that a portion of the light absorbed by the chlorophyll goes to warm the chlorophyll-grain, and hence the surrounding parts also; so that even if we accept Stahl's view, the absence of the red dye from the guard-cells may simply be due to the fact that the greater amount of chlorophyll which they contain enables them to dispense with any special and very much less efficient heat-absorbing medium such as erythrophyll.

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[1] Wiesner, Sitzungsbericht d. K. Akad. der Wiss. zu Wien, Bd. lxxiv. p. 1876.

That the stomata are markedly sensitive to and may be injured by prolonged exposure to sunlight, so that the presence of a protective red dye in the guard-cells might possibly under given conditions of exposure be an advantage to the plant, is shown by the following observations. The adult leaves of a number of plants having stomata on both surfaces were exposed to bright sunlight, in some cases the upper and in others the under surface being the exposed one: the epidermis was then removed and examined under optimal illumination by the Bacterium-method to ascertain whether the assimilatory powers of the chlorophyllous guard-cells were affected in any way. The guard-cells are rather sensitive to the action of prolonged sunlight; and often four to five hours' exposure, especially when it is the under surface which is exposed, is sufficient to inhibit their assimilatory activity, the power as a general rule returning again in from a few hours to a day, if placed under conditions which permit of recovery. With longer exposure, and especially if subjected to the cumulative effects of exposure extending over several days, the chlorophyll-grains are more or less completely bleached, and may be in a condition of permanent light-rigor from which no recovery is possible. It is commonly the case that in exposed leaves which have been for some time adult but which are still quite vigorous, no power of assimilation can be detected in the guard-cells of the stomata, if any, on the upper surface: and this may still be the case, especially in well-exposed leaves, even if previously kept under conditions which permit of recovery, the chloroplastids in such cases being in a condition of permanent light-rigor. The guard cells from the under surface of normal leaves, when examined in the morning, almost without exception show a more or less marked power of assimilation and this persists until the decline of the leaf commences

Bearing these facts in mind, it is easy to understand that, in much exposed and horizontally expanded bifacial leaves, it should be an advantage to the plant to have the stomata restricted entirely or almost entirely to the under surface of the leaf; whereas in an erect leaf, or one growing in the shade, the stomata tend to be more evenly distributed over both surfaces. It is interesting to notice that in a bifacial leaf, with more than one layer of palisade-parenchyma, owing to the stomata being more abundant on the under surface of the leaf, the amount of assimilation performed by the different layers of the palisade-parenchyma tends to be equalized under normal conditions of exposure and illumination, the deeper layers coming into contact with air containing a higher percentage of CO_2[1], whilst the upper ones are exposed to stronger illumination.

Stahl mentions that the marked red colouration which the stigmas of many anemophilous plants show, may be for the purpose of raising the temperature of the stigma, and hence aiding the growth and germination of the pollen, on which, according to Stahl, light exercises no perceptible influence. In a previous paper[2] it has however been shown that exposure to light of marked intensity exercises a distinct influence on the germination of the pollen-grain, and more especially retards the growth in length of the pollen-tube. Hence, considering the generally much exposed condition of the stigmas of anemophilous plants, it is very evident that the red dye even in this case may be of paramount importance as a protection against the inhibitory action which strong light exercises on the growth and elongation of the pollen-tube.

In the case of alpine and arctic plants, the slight additional absorption of heat which the red dye causes, may be of some use; but in all cases, other things being equal, an increased absorption of heat means an increased transpiration, which, in alpine plants without a marked xerophilous[1] habit, might be distinctly injurious, owing to the difficulty of absorbing water from the cold ground in sufficient amount to cope with the rapid evaporation caused by the low pressure and dryness of the air. Besides, the insolation-temperature in alpine regions is already sufficiently high: it is at night and when shaded that an absorption of heat might be of considerable use.

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[1] Blackman, On the Path of the Gaseous Interchanges in the Leaf: Proceedings of the Royal Society, 1896.

[2] Observations on the Pollen-tube: Trans. Liverpool Biol. Soc., Vol. ix, 1895.

In *Haematococcus* and similar forms the light-protecting function of the red dye is probably the most important one; for, as Schröder[2] and Klebs[3] have shown, the common presence of a red dye in the resting-spores of many Algae is of importance as a protection against the decomposition to which the chlorophyll in such cases is especially liable when exposed to light. Thus the green zygotes of *Hydrodictyon* may remain living if dried in darkness, but if exposed to light become decolorized and die.

Stahl considers the fact that the red colouration, which the young shoots of plants growing in cold climates generally show in spring, commonly disappears on the advent of the first few warm days, as being conclusive evidence pointing to the red dye being of importance as a means of raising the temperature of the young growing parts. It is however just when the temperature is low that the chlorophyll-grain is most liable to the decomposition induced by the action of light[4], and when the light, if sufficiently intense, most markedly retards the development and turning green of the etiolated chloroplastid.

If plants of *Elodea canadensis*[5] or *Utricularia vulgaris* are immersed in weak sugar-solution kept at ordinary room-temperature and exposed to strong light, they may show a marked tendency to form a red dye, whereas in water or diffuse daylight but little or none is formed. Hence under conditions in which the chlorophyll-grain tends to become unhealthy and its assimilatory activity to become inhibited[1], a red dye protecting it from the effects of excessive exposure to which it is now specially sensitive, tends to be formed. The non-formation of the red dye under exposure to weak diffuse daylight, is strong though not conclusive evidence that the red dye is not merely an accidental metabolic product formed under these peculiar conditions of nourishment. Obviously, since the plants are submerged, it cannot possibly be for the purpose of increasing what is non-existent, i.e. transpiration, nor can it perceptibly raise the temperature of a submerged water-plant. When a red colouration is present in underground parts (Beet-root, &c.) which are not exposed to light or only to very faint illumination, it may very possibly be a waste metabolic product accidentally produced, and perhaps without any special function.

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[1] Lazniewski, Beiträge zur Biologie der Alpenpflanzen: Flora LXXXII, 1896, pp. 224-267.

[2] Schröder, Bot. Untersuch. Tübingen, Bd. ii, Hft. 1, 1886.

[3] Klebs, Bot. Untersuch. Tübingen, 1883, Bd. i, Hft. 2.

[4] See Assim. Inhibition: Journal Linnean Society, 1896, p. 390 (Coniferae).

[5] Assim. Inhib.: Journal Linnean Society, Vol. xxxi, 1897 (Botany), p. 567.

J. C. Costerus[2] finds that in most tropical plants before 6 a.m. an abundance of starch is present, that it decreases rapidly until 7 a.m., then increases again and reaches a maximum in some plants at noon, and in others not until 4 or 5 p.m. During the night but little starch is removed, and Costerus concludes that light probably exercises a most important influence in favouring the translocation of carbohydrates. Pick[3], on the other hand, long ago came to the conclusion that light inhibits or retards the translocation of carbohydrates, and that the red dye is developed as a protection against this action of light. Pick's interpretation of the function of the red dye has already been shown[4] to be only a special and less important case of its general protective function. The slow removal of the accumulated starch at night, and its rapid diminution in the early morning when exposed to light, are not necessarily due to any direct

[1] Loc. cit. footnote 5, p. 477.

[2] Costerus, Ann. d. Jar. Bot. de Buitenzorg, p. 73, Vol. xii, Pt. I, 1894.

[3] Pick, in Bot. Centralblatt, XVI, pp. 9-12.

[4] Linnean Soc. Journal, 1896 (Bot.), p. 445.

influence of light on translocation, but may be indirectly due to the light causing a markedly increased transpiration, and so increasing the velocity of the conduction-currents as to rapidly remove the soluble carbohydrate as fast as it is formed, thereby permitting of the rapid solution and removal of the accumulated starch. At the same time it is in the early morning, as the temperature rises, and before the light becomes too strong, that growth is most active, and the stored material is most rapidly used up.

There can be little doubt that, both in the tropics and in temperate climes, the main and primary function of the red dye, when present in exposed parts, is to act as a protection against light of too great intensity; though in all cases its presence at the same time confers upon the plant a slightly increased power of absorbing heat. For calling attention to this latter possibility Stahl deserves full credit from both the physiologist and the biologist: in a few cases, such as in the horizontal leaves of shade plants having the red colouration present on the under surfaces only, the relatively slight heat-absorbing power of the red dye may, by secondary adaptation, have become its most important function.

SUMMARY.

In tropical plants full and prolonged insolation may markedly affect or temporarily inhibit the functional activity of the assimilating parts. If the stoppage be temporary, it is generally accompanied by but little change in colour; but if permanent, the colour of the chlorophyll-grains is markedly affected, and they may be completely bleached. Different plants show very different resistant powers, shade-loving plants being, as might naturally be expected, the least resistant.

The leaves are protected against the injurious effects of excessive exposure by the presence of a red dye and by active or passive paraheliotropic movements. The active movements are best shown by the motile leaves of the Leguminosae, the pulvini being the motile and irritable perceptive organs. In *Mimosa pudica* the irritability is very marked and well differentiated, the main pulvini responding to the direction of the light in a diaheliotropic manner, the pulvini of the leaflets responding to the intensity only of the illumination, being paraheliotropic in intense light, diaheliotropic in diffuse daylight, and nyctitropic in very weak light or darkness. It is mainly to the photochemical rays, which are most active in inducing the decomposition of chlorophyll, and in inducing light-rigor, that the pulvinus of the leaflet responds.

The red pigment acts mainly and primarily as a protective shield against the more refrangible rays (green and blue). It has also a feeble heat-absorbing power, which may, in a few cases, possibly be of considerable or even primary importance.

NOTES.

THE GAMETOPHYTE OF BOTRYCHIUM VIRGINIANUM.—At the suggestion of Dr. Scott, the following note on the gametophyte of *Botrychium virginianum* has been prepared by the writer.

The account is slightly modified from an abstract published in the proceedings of the Canadian Institute, Vol. i, Part I, 1897. A full description with the necessary plates and references to the literature will shortly appear in the Transactions of the Canadian Institute.

A complete description of the gametophyte of the Ophioglossaceae has long been a desideratum. Since the discovery by Mettenius, in 1856, of the subterranean prothallium of *Ophioglossum pedunculosum*, and by Hofmeister, in 1857, of that of *Botrychium Lunaria*, nothing has been added till recently to their necessarily incomplete accounts of the gametophyte in these species. Our latest knowledge on this subject is derived from a brief description of rather advanced material of the prothallium of *Botrychium virginianum* found in 1893 at Grosse Isle, Michigan, by Professor Douglas Campbell, which was published in the proceedings of the Oxford meeting of the British Association in 1894.

A more extended description of the same material, together with an account of the first stages in the germination of the spores of *B. virginianum* and *Ophioglossum*, appeared in his 'Development of Mosses and Ferns' (1895). These prothallia did not, however, supply the stages in the development of the sexual organs and the sporophyte.

During the summer of 1895, the writer secured a large number of prothallia of the same species at Little Metis in the Province of

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Quebec. On examination it was found that the material thus obtained afforded a complete elucidation of the development and structure of the antheridia and archegonia, and a less satisfactory series of stages in the segmentation of the embryo. Last summer the remaining prothallia were removed, to the number of about six hundred, and although they have only been partially studied yet, owing to technical difficulties in embedding them, those examined have supplied all the lacking stages of the development of the young sporophyte.

All the younger prothallia were found in a single circular depression of Sphagnum-moss about ten feet in diameter, near a corduroy road, running through the wooded margin of a peat and huckleberry swamp at Little Metis, P. Q. Older prothallia were abundant with those bearing fertilized and unfertilized archegonia and younger embryos.

I have also found young sporophytes of several years' growth in the woods on the heights back of Metis; in the 'Flats' below the 'Whirlpool' on the Niagara river, and also in rich woods along the valley of the Don, near Toronto. In all the examples last referred to the young spore-plant was still attached to the gametophyte. It seems probable that the prothallia of our common Canadian species of *Botrychium* are much more easily obtainable than has been hitherto supposed. It is necessary to add, however, that although my attention has been directed to the subject for some three years past, I have not yet succeeded in finding the younger stages of the prothallia in any other spot than the Sphagnum-basin in the swamp at Little Metis.

The gametophyte of *Botrychium virginianum* is of flattened oval shape, the narrower end of the prothallium being terminated by the growing-point. My specimens are from two to eighteen millimetres in length, by one and a half to eight millimetres in breadth. Their thickness increases from the growing apex backwards. The sides and lower surface of the prothallium are covered in younger specimens with multicellular hairs. In older plants these tend to disappear. The middle of the upper surface is occupied by a well-defined ridge, upon which the antheridia are situated. The archegonia are found on the declivities which slope away from the antheridial ridge.

As might be expected, the younger sexual organs are found nearer the growing-point than those of greater age.

A cross-section of the prothallium reveals to the naked eye the fact that the lower part of the gametophyte is composed of tissue which is yellowish in colour, and from which a thick oil exudes, even when the plant has been lying in ninety per cent. alcohol for months. The upper portion of the prothallium-tissue, upon which the generative organs are situated, is white in colour and free from oil. A longitudinal section of the prothallium shows the same distribution of yellow oil-bearing and white oil-free tissue as the cross-section, but demonstrates that the oil-bearing stratum is both absolutely and relatively much thicker in the older parts of the plant.

Microscopic examination shows that the oleiferous tissue has its cells occupied by an endophytic Fungus and a very abundant protoplasm.

The Fungus, so far as it has yet been studied, seems to be a sterile *Pythium*, possibly the same as that found by Treub, Goebel and others, in the prothallium of species of *Lycopodium*. The writer hopes to investigate the Fungus more closely in a living condition during the next period of vegetation. The Fungus-filaments can be seen passing from the prothallium to the outside medium by way of the root-hairs.

The prothallium seems to be entirely saprophytic in its mode of life; as quite young examples, bearing as yet only antheridia, were found, which were nevertheless yellow in colour and wholly subterranean. They showed no evidence of a scar, indicating a possible origin from a green subaerial phase, even when examined under considerable magnification; and in fact the depth of their occurrence in the moss (in many cases 10 cm. or more) would seem in itself to preclude such a mode of origin. Moreover, Mettenius found in the case of *Ophioglossum pedunculosum*, that the subterranean saprophytic stage was antecedent to the green lobes, appearing above the soil. Somewhat similar conditions have been described by Treub in species of *Lycopodium*.

Campbell describes the appearance of chlorophyll in the germinating spores of this species, but it may have originated from the spores being sown, contrary to the natural conditions, in the light. The writer is experimenting with growing spores in darkness, but sufficient time has not yet elapsed for germination to take place.

The antheridia, as has been already stated, occur in numbers on a ridge running lengthwise on the upper surface of the prothallium. The young antheridia originate behind the growing-point from a single superficial cell. This divides transversely, the outer half giving rise to the outer antheridial wall and the inner half by repeated simultaneous divisions to a large number of spermatocytes. The fully developed antheridium is largely embedded in the antheridial ridge, and projects only slightly above its surface. The formation of the spermatozoids has not yet been carefully studied, but seems to resemble closely that described in the Marattiaceae and Equisetaceae. The spermatozoids are usually large in size, but otherwise resemble the ordinary Fern-type, and consequently differ from the biciliate Moss-like spermatozoids of the Lycopodiales.

The archegonia are confined to the sloping sides of the upper surface of the prothallium. Unlike the antheridia, young archegonia, although most abundant near the growing-point, may be formed on almost any part of the archegonia-bearing surface. The mother-cell of the archegonium is superficial, and is distinguished from its neighbours by a large nucleus and a more abundant protoplasm. It first divides transversely into a shallow outer cell and a deeper inner cell. The inner cell divides again, and as a result the young archegonium consists of three cells. The most external of these, by subsequent divisions, give rise to the neck of the archegonium. The internal cell is the basal cell. It also divides into a plate of cells, sometimes composed of two layers, distinguished by their richly protoplasmic contents. The middle cell of the young archegonium-series gives rise by division to the neck-canal-cell and to the ventral cell. The former becomes binucleate, but never divides into two cells. The latter, just before the maturation of the archegonium, divides into the egg-cell and the ventral canal-cell. The ventral canal-cell is broad, like that of the Marattiaceae.

In the ripe archegonium, the nuclei of the cells of the upper stories of the archegonium-neck become chromatolysed. I do not know yet whether this feature is peculiar to *Botrychium*.

The fully-developed archegonium is sunk into the prothallium, and only the neck projects above its surface. The cervical cells are in four rows as in the other Pteridophyta, and the terminal ones spring apart when the egg is ripe.

Spermatozoids are frequently found in contact with the egg. After fertilization the egg grows to many times its original size, and the reduced protoplasm contains a large hydroplastid.

The first division of the oospore is across the long axis of the archegonium. The next division is parallel with the long axis of the prothallium, and at right angles to the first. The third cross-wall is in the transverse direction of the prothallium, and at right angles to the other two. I have been unable to follow satisfactorily the subsequent divisions. The organs appear very late, and only after the embryo has attained a large size. The root is the first of them to emerge, and the proliferation of the cells, indicating its place of origin, is long unmarked by the presence of an apical cell. The cotyledon, stem-apex, and the foot, appear nearly simultaneously. The root and cotyledon originate from the upper part of the embryonic mass; the foot and stem-apex from its lower cells. The apex of the root in many cases is in the same straight line with the canal of the archegonium-neck.

It seems hardly possible to derive the organs from definite octants of the embryo.

The growth of the root ruptures the calyptra, and its exit is followed somewhat later by that of the cotyledon. The latter is not a bilaterally symmetrical structure, as in most Ferns, but is of the same palmate type as is found in the Osmundaceae. The cotyledon begins to assimilate as soon as it reaches the surface of the ground, and thus resembles that of *Ophioglossum pedunculosum*.

There seems to be no evidence to indicate that more than the cotyledon appears above ground in the first season of the young plant's growth. In following summers apparently only a single leaf is produced, as is the case with the older plant. I have found young sporophytes, bearing their sixth leaf, still attached to the mother-prothallium; and as I have never found more than one leaf on the young spore-plants at once, and as the leaves, like other organs of this species of *Botrychium*, are extremely resistant to decay, I am reasonably certain that such examples were in the sixth year of their existence. This longevity of the gametophyte is of some interest.

One frequently finds two or more sporophytes on a single prothallium, and in many of these cases the apex of the prothallium is bifurcated. In one case I found two spore-plants which had arisen from a single embryo. In another case I discovered two tracheids in a prothallium in the vicinity of a decayed young spore-plant. The latter may have been of apogamous origin, as a similar phenomenon generally accompanies apogamy. I have not yet studied thoroughly the growing region of the prothallium, as it is best examined in longitudinal sections of the gametophyte. So far as I have investigated the matter, there seems to be evidence of the existence of an apical cell.

E. C. JEFFREY.

UNIVERSITY OF TORONTO,
May 28, 1897.

BACTERIA WITH ASSIMILATORY PIGMENTS, FOUND IN THE TROPICS.—The following Bacteria, having a greenish colouration and showing when exposed to light a faint evolution of oxygen, perceptible by means of motile Spirilla or Micrococci and very exceptionally causing *Bacterium termo* to faintly re-act, were found in water-cultures of more or less purity developed in diffuse daylight at Buitenzorg: viz. a motile green Bacterium = *B. chlorinum* (Engelmann)[1]; a non-motile Micrococcus-form to which the provisional name of *Streptococcus varians*[2] has previously been given; two forms closely resembling Van Tieghem's *Bacillus virens* and *Bacterium viride*[3]; two green Spirilla, one resembling *S. tenue* and the other *S. undula*; and finally a large Bacillus-form somewhat resembling the *Bacillus virens* of Van Tieghem. This last form occurs as short rods, 2·5 to 3 μ broad and commonly 12 to 15 μ, more rarely 5 to 20 μ long. A formation of colourless refractile endosporous spores is often shown. The spores are oval and 1·5 to 2 μ broad by 2 to 3·5 μ long. In all cases the pigment is diffused throughout the plasma of the bacteroid-cell, and this is especially clearly shown in the large *Bacillus virens* and *Spirillum undula* forms.

Of the two more common red water-Bacteria, *Monas okenii* was not found in either Java or Ceylon, but *Bacterium photometricum* was. In Java *B. photometricum* appears to be abundant and widely distributed. In water-cultures exposed to diffuse daylight, the Bacteria collect in the form of a red crust upon the walls of the cylinder. By removing this crust a large mass of the red *Bacteria* may be obtained; which if the growth is of recent formation may be nearly pure, and, what is of more importance, almost entirely free from other coloured Bacteria or Confervae. If to the brownish red mass thus obtained alcohol is added, the resulting fluid is reddish in colour, turning to a dark dirty green on warming. If ether is now added and the mixture then diluted with water and shaken, the ether separates and rises to the surface as a dark bluish green fluid, showing a red fluorescence, whilst the fluid beneath is colourless and contains a white flocculent residue. As the ether evaporates, a pinkish red dye which it also holds in solution, but which is masked by the green dye, is deposited in rings at the edges and on the sides of the tube or evaporating basin. If, instead of using ether, the separation of the green dye from the warmed alcoholic extract is effected by benzene, the fluid beneath remains a pinkish red, whilst the supernatant benzene is dark bluish green in colour, and shows a red fluorescence in reflected light. The alkali and acid methods[1] of extraction for alkachlorophyll and chlorophyllan yielded brownish fluids only. Both the benzene and the ether extracts, when exposed to light and in the presence of oxygen, rapidly become brown and fade. It is true that the material from which these extractions were made was not absolutely pure, microscopical examination before extraction showing the presence of occasional green organisms; and even though these formed but a fraction of a percentage of the total mass, still for absolute certainty the extraction needs to be repeated with perfectly pure cultures, which, owing to the peculiar conditions under which *B. photometricum* develops, and the consequent extreme difficulty of isolating it, are by no means easy to obtain in sufficient quantity. Still as far as they go the facts above mentioned seem to indicate that a green dye apparently identical with chlorophyll may be extracted from *B. photometricum*, as well as a pinkish red one which is insoluble in benzene but soluble in ether, and in alcohol even when diluted largely with water. Engelmann[2] has shown that the point of maximal assimilation in *B. photometricum* corresponds with the point of maximal absorption, which curiously enough lies, as determined by his bolometer experiments, in the dark ultra-red rays. It appears, therefore, that *B. photometricum* resembles the Florideae in so far as the bacterio-purpurin which it contains is a compound assimilatory chromophyll, which when treated with hot alcohol splits up into two differently coloured substances, namely, chlorophyll and a pinkish red pigment which in colour and solubility shows a distinct resemblance to the pinkish red pigment which may be extracted from red Algae.

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[1] Engelmann, Zur Biologie der Schizomyceten, Bot. Zeit. 1882.

[2] The Evolution of Oxygen by coloured Bacteria: Journal of the Linnean Society, 1897.

[3] Van Tieghem, Bull. Soc. France, XXVII, 1880, p. 174.

A. J. EWART.

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[1] See Marchlewski, Chlorophyll and its Derivatives.

[2] Engelmann, Bot. Zeitg., Oct. 1888.

STUDIES IN THE MORPHOLOGY OF SPORE-PRODUCING MEMBERS: PART III. MARATTIACEAE. By F. O. BOWER, Sc.D., F.R.S., Regius Professor of Botany in the University of Glasgow[1].

THE memoir, of which this is an abstract, deals with the sori of all the four living genera of Marattiaceae; the development has been traced in *Angiopteris* and *Marattia* from the earliest stages to maturity, in *Danaea* and *Kaulfussia* from such early condition as the material would permit. Some of the results from *Danaea* have been already submitted to the Society in a preliminary statement[2]. One result of the investigation has been to demonstrate, as regards their development, the substantial unity of type of the sporangia in the four genera. In all of them a single 'superficial parent cell' of prismatic form is to be recognized embedded in the massive sporangium when young, not in a central position, but directed obliquely towards the centre of the sorus. By periclinal division this forms internally the archesporium, externally that part of the wall where dehiscence takes place. The tapetum arises, typically in them all, from the cells surrounding the archesporium. The dehiscence is in all by a slit in a radial plane, which may widen to a circular pore in *Danaea*. In those sori where the sporangia are united laterally there is no annulus; it is present only where the sporangia are separate, as in *Angiopteris*.

An interesting feature is disclosed by estimates of the potential spore-production of the single average sporangium in the four genera; the results in round numbers are, in *Angiopteris* 1,450, in *Danaea* 1,750, in *Marattia* 2,500, in *Kaulfussia* 7,850. It is to be remembered that the usual numbers in Leptosporangiate Ferns are 48–64; in some Leptosporangiate Ferns (*Osmunda*) the number may rise to 500. I have ascertained in *Gleichenia*, however, that the number may be as high as in *Angiopteris*. This large potential output of spores goes parallel with the broad base of the sporangia; in fact, the Eusporangiate condition is that best adapted for maturing large numbers of spores in the individual loculus.

Frequent deviations from the type have, however, been observed, as well as variations of size and mode of segmentation of the sporangia, and it is not possible in certain cases to refer the whole sporogenous tissue of one sporangium to a single parent cell. A special study of the irregularities has been made in *Danaea*, in which genus they are most marked; incomplete septa are frequent, and the sporangia are of very unequal size. The main features have already been noted in the preliminary statement on that genus, where it has been pointed out that comparison of the details with those of the septate anthers of some Angiosperms shows that there is a remarkable resemblance between the two cases. Similar irregularities have been noted, though less commonly, in *Kaulfussia*, and *Marattia*, and rarely in *Angiopteris*.

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[1] Abstract of a paper read before the Royal Society, June 17, 1897.

[2] Roy. Soc. Proc., vol. lix. p. 141.

Those fossil Marattiaceae which are best known as to the details of the sorus have been compared, and the substantial similarity of the sori in certain cases to those of the modern genera recognized. The facts from fossils and from the modern Marattiaceae have been made the basis for a fresh discussion of the theoretical question, whether the synangium is or is not a result of coalescence of sporangia? It is concluded that the palaeophytological evidence leaves the question open as to the priority of existence of forms with synangia, or with separate sporangia, in the Marattiaceae. Notwithstanding that writers of authority have treated the question as decided, that the synangia are a result of fusion of distinct sporangia, it is held with some persistence that it is still open; the palaeophytological evidence is inconclusive, while the comparative evidence from the living genera will not only accord with, but appears actually to support a view of septation.

For the analogy with septate anthers, where septation must have occurred, and the similarity between the details of these and those in *Danaea*, and especially the partial septations in both, make it appear probable that in this genus progressive septation has taken place. It is thought probable that progressive septation has been a feature, at least where the sori are elongated, as in *Danaea*. But the question is left over for future discussion whether or not a similar septation, rather than coalescence, may be accountable also for the origin in the first instance of a circular sorus with a plurality of sporangia united together as in *Asterotheca*, or in *Pecopteris unita*.

The Movement of Protoplasm in Coenocytic Hyphae[1].

BY

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With Woodcuts 8, 9, 10, 11.

THE translational movement of protoplasm varies much in the manner of its appearance, but for the most part may be considered under three headings: viz. circulation, as in the staminal hairs of *Tradescantia*; rotation, as in cells of *Chara*; and streaming, as in plasmodium of Myxogastres. There is, beside, the sliding movement seen in Desmids, and the pulsating movement of many locomotive organisms, and others less common. All these are undoubtedly spontaneous movements, and together with such induced movements as the orientation of chlorophyll-grains in leaf-cells of *Oxalis* and *Lemna* and in filaments of *Mesocarpus* and *Caulerpa*, when acted upon by light, are probably conditional upon some property of living matter not yet understood, which acting under diverse conditions brings about different manifestations of energy.

Of the several forms of movement, streaming is most pronounced, as the mass involved is larger and the speed

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[1] Read before the Botanical Society of America at the Toronto Meeting, August 18, 1897.

[Annals of Botany, Vol. XI. No. XLIV. December, 1897.]

greater. Its discovery in hyphae is therefore of considerable interest. So far as I know, literature shows but few references to the streaming movement of protoplasm in hyphae. One occurs in Vines' 'Textbook of Botany' (1895), where (p. 735) it is cited in a general way as one of the several instances of protoplasmic movement; and on the following page the direction of such movement is said to be various, except 'in plasmodia and in hyphae,' where it is a 'simple longitudinal movement.' A more definite account of the movement in hyphae is given by De Vries[1] in the Botanische Zeitung for 1885, in the case of the sporangiophores of *Phycomyces*. At the time of spore-formation the contents of the sporangiophores of this species appear to pass into the sporangia. The sporangiophores are very long, occasionally exceeding 30 cm., and unusually thick, and movement within them can be easily observed. De Vries appears to have considered this a special instance of protoplasmic movement, clearly designed for rapid supply of water and nutriment to the forming sporangia and not necessarily of the same nature as the movement in *Tradescantia* and the Myxogastres[2]. This interpretation is strengthened by the fact that he makes no reference to it in the revised edition of his *Leerboek der Plantenphysiologie*, issued a few months later, although eight pages are given to a careful presentation of protoplasmic movement.

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[1] De Vries, Hugo, 'Ueber die Bedeutung der Circulation und der Rotation des Protoplasma für den Stofftransport in der Pflanze.'—Bot. Zeit., 43: 1–6, 16–26.

[2] De Vries' words are as follows:—'Betrachten wir zunächst einen besonderen Fall, in welchem die Bedeutung der Bewegung des Protoplasma für den Stofftransport klar zu Tage tritt. In den Fruchtträgern von *Phycomyces nitens* bewegt sich das Protoplasma von einem Ende bis zum anderen mit einer solchen Geschwindigkeit, dass die ganze Länge des Fruchtträgers von den einzelnen Theilchen des lebendigen Inhaltes in wenigen Stunden durchlaufen wird.'—l. c. page 4.

'Es ist klar, dass in manchen Fällen nicht nur die eigentlichen Nährstoffe, sondern auch das Wasser, nur durch die Strömung des Protoplasma in genügender Weise transportirt werden kann. Solches gilt u. A. offenbar von den Fruchtträgern von *Phycomyces* und den Wurzelhaaren der höheren Pflanzen.'—l. c. pp. 25–26.

My attention was first called to movement in hyphae in 1890, while examining bacterial cultures on plates of nutrient gelatin which had been invaded by some Mould, afterwards ascertained to be *Rhizopus nigricans*. Some account of these early observations was given before the Indiana Academy of Sciences at the meeting in December of that year[1], but nothing was recorded in print regarding the matter. The movement was so very striking, and in such very common objects, which had been favourite subjects of study by systematists, morphologists and physiologists for a century or more, that I hesitated to believe that it was unknown to science.

For a very long time the conditions under which the movement takes place were not recognized, and it was only seen at intervals, and then for so brief a space of time that serious study was impracticable. In course of time it was found out that the most important condition is a saturated, or nearly saturated, atmosphere, and all subsequent studies were made with the cultures covered so far as possible.

Some time spent at the Botanical Institute at Bonn during the summer of 1896 enabled me to prosecute my inquiry into the facts, and also to satisfy myself that no record of them had yet been made. I take the opportunity to acknowledge the kindness of Professor Strasburger in placing the resources of his laboratory at my disposal, in providing additional apparatus for my work, and of both Professor Strasburger and Dr. Noll for helpful suggestions.

The hyphae of the Mucoraceae after attaining a certain maturity, I have found, set up a movement of the protoplasm, in which nearly or apparently all the contents—that is, the cytoplasm, microsomes, food-bodies, nuclei, and vacuoles—participate. It is a streaming movement, such as a somewhat viscous, colourless liquid would produce in flowing through a pipe. There is sometimes an evident ectoplastic layer lining the cell-wall that does not take part in the movement, but often this is so thin that it is no longer visible, although one cannot suppose that it is ever entirely absent. All kinds of granules are borne along in the current. The vacuoles, however large, are also swept along; and at the right stage of growth for movement the protoplasm is usually highly vacuolated.

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[1] Bot. Gaz. xvi, 36.

The movement is usually fitful. It does not take place in all the hyphae of an individual plant at the same time, but occurs in some of the main filaments and in part of the branches leading therefrom. It continues for a time, then without apparent reason ceases. Again it starts, either in the same direction, or more usually the opposite. The periods of movement and of rest are both indefinite. The current may flow in one direction for fifteen or twenty minutes, or possibly much longer, without the slightest check, and with brief interruptions may continue for hours.

The observer is soon curious to know where so much protoplasm and cell-sap goes to. If a hypha in which the current is flowing distally be traced to the end, it will be found that the current finally becomes slower, but does not cease until the end is nearly reached. Nothing is revealed to the eye, however, that explains how the full hyphal extremity continues to receive an inflowing stream without seeming limit. We might liken it to a small lake with no outlet, into which a rapid river flows without effecting a change in the level. It would seem that the end of the hypha should be under greater stress than the part further back; but there is no clear evidence of it in change of diameter, elongation, or extrusion of water.

In tracing the filament containing the current from the free end towards the centre of the mycelium, I have always found that sooner or later it became entangled in the general mass and was lost to sight. A more ingenious method of growing the Mould may eventually enable the observer to map out the currents throughout the diversified ramifications of a whole plant.

The rate of movement varies greatly, and rarely remains at a uniform speed for many minutes at a time. I made a number of readings of rapidly flowing protoplasm in

Rhizopus nigricans at a temperature of 28° C., and found it to average 5·5 mm per minute. This is about twice as fast as the rotation in *Nitella* and four times that of the circulation in *Tradescantia*. It does not, however, equal the rate of streaming in the Myxomycetes, as given by Hofmeister[1].

[illegible]

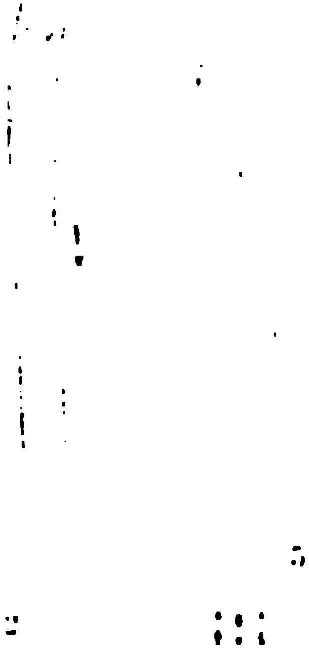
[illegible] in the capsules of [illegible]

[illegible]

No direct observations were made regarding temperature, but incidentally the unusual range for rapid movement attracted attention. Cultures grown in the refrigerator, standing at about 10° C., and transferred directly to the stage of the microscope, showed as rapid movement apparently as those grown at 30° C. Not only does the organism appear less sensitive in this respect to the range of temperature than in most other known instances, but it is not readily affected by sudden change, shock, and other stimulating agents, judging from the limited number of observations made.

A matter of considerable interest in connexion with this study is the behaviour of the moving vacuoles. Sometimes they are few and small, and when at rest are as usual globular. More frequently they are numerous, and many of them so large that the confines of the hyphal tube distorts them into long cylinders with convex ends. Occasionally a hypha will be so filled in places with vacuoles that only a very thin lining of protoplasm clings to the wall-surfaces, and at long intervals a plate of protoplasm extends across the lumen. Under all these variations movement takes place with seemingly equal readiness. In a rapidly moving current the vacuoles become more convex at the anterior end, and less convex, flat or even concave, at the rear end. A very long vacuole is usually preceded by dense protoplasm, into which it seems to push, and is followed up by a mass of vacuoles with such thin walls that they have the appearance of foam. The way in which the foremost and thinnest-walled ones change position, vary their form, and coalesce, as they are swept along, reminds one forcibly of the behaviour of soap bubbles (Fig. 2).

Very interesting changes occur in the form and individuality of the vacuoles when the stream passes through a bent and tortuous filament, or over an obstruction such as may be produced by doubling the hypha upon itself so sharply as to nearly close the passage, or when two streams flow together, as may happen at any angle from a very acute to a very obtuse one, or when a stream sends part of its contents into a side branch, or when a rapid stream forces itself upon and merges into a slower one. I shall, however, content myself at this time by saying that these appearances are such as would be produced by a viscous fluid, holding



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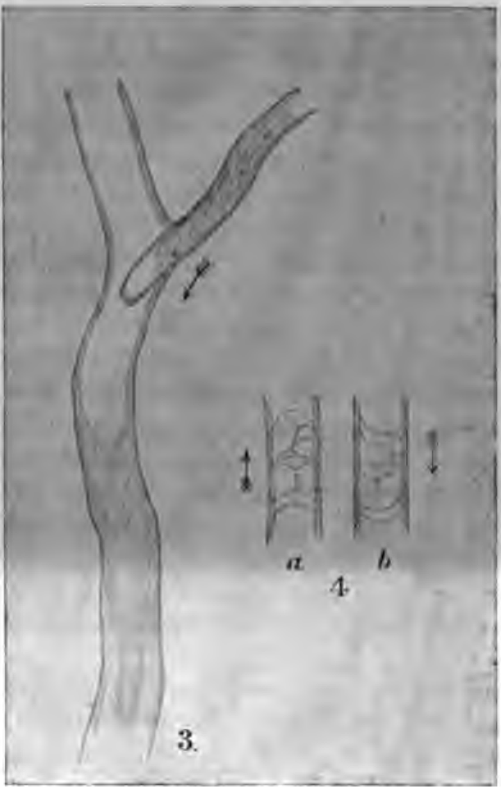
[illegible] when forced through tubes of like [illegible] filaments. When, for instance, the stream [illegible] separately upon the angle of a wall separating two branches, the larger vacuoles are generally bisected, a part of each going to the right and a part to the left. When one stream flows into another, it frequently plunges into the midst of large passing vacuoles, and disruptures them. When a part increases its speed over the part immediately preceding, better progress is made by pushing



WOODCUT 10.

Fig. 3. The current from a branch is rushing into a long vacuole in the main hypha. Further along the main hypha, the protoplasm is pushing into and through a large vacuole (only partly shown), owing to an acceleration of the current.

Fig. 4. Portion of a highly vacuolated hypha with the protoplasm in motion, *a* just before, *b* just after, a change in direction of the current.

through the centre of a long vacuole, as this lightens the friction at the sides (Fig. 3).

In fact I cannot see in the variety of catastrophes which overtake the moving vacuoles any ground of support for the supposed autonomy of the vacuoles and a special vacuolar membrane, advocated by De Vries[1], Went[2], Wakker[3], Bokorny[4] and others. These theories appear to me to be little in accord with the phenomena that attend the streaming in coenocytes, while much can be seen to favour the views of Pfeffer[5], Bütschli[6] and others, who appeal to the laws governing viscous fluids in which surface tension assumes an important rôle. But my observations have not been sufficiently intimate and complete to warrant me in pursuing this phase of the subject.

A requirement of the first importance for the display of movement, as I have said, is an atmosphere heavily charged with moisture.

Unless extravasated drops are plentifully scattered over the hyphae, movement is not likely to occur. It will probably be interesting to describe an instance of their origin and subsequent behaviour as seen under a Zeiss microscope with D objective and No. 4 ocular (Woodcut 11). The culture was on nutrient gelatin with *Rhizopus elegans*. A filament lying in contact with the substratum and showing vigorous streaming was traced to the growing apex, and found to project into the air. The proximal half of the aerial part was plentifully supplied with drops. The distal part was free from drops. The observations began at 4 p.m., and twenty minutes afterwards drops began to appear at some distance apart. These grew larger, and intermediate ones appeared. At 5 o'clock branches began to protrude through the larger drops. In half an hour the branches had grown quite long, and they themselves began to show drops, which by 6 o'clock became plentiful. In the meantime the proximal half had increased and enlarged its drops, but no branches had appeared. The protoplasm had streamed into the portion under observation almost continuously for a time, then at intervals it stood still, the intervals of rest becoming longer and longer, but no reverse movement occurred. Observations were continued until 7 p.m., three hours in all. During this time the branches grew at an apparently uniform rate, and without noticeable synchronous relation to the protoplasmic movements. The next morning observations were resumed, and from 10.30 to 11 a.m. streaming

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[1] Pringsh. Jahr. f. wiss. Bot. xvi, 463. 1885.

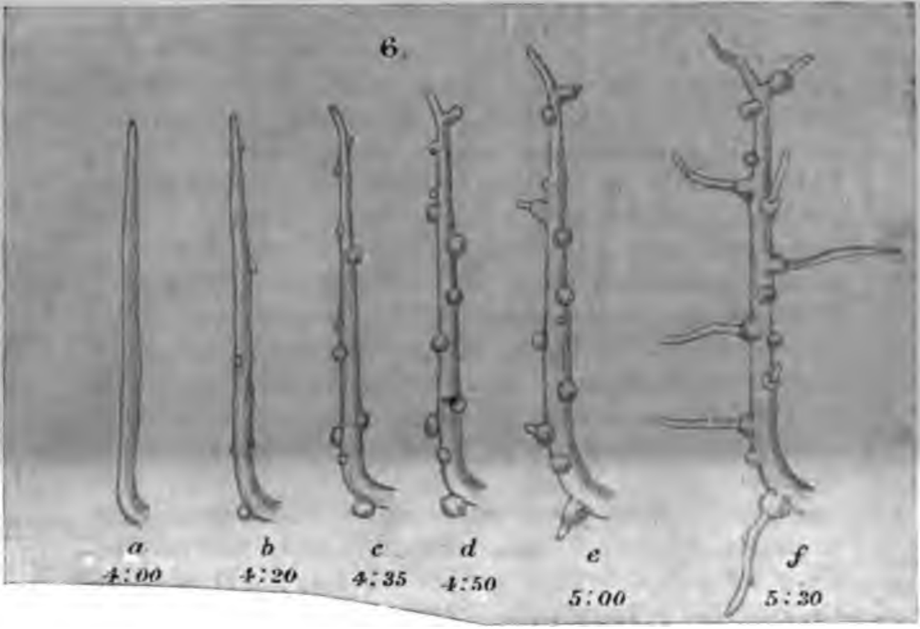
[2] Ibid. xix, 295. 1888.

[3] Ibid. xix, 423. 1888.

[4] Biolog. Centr. xiii, 271. 1893.

[5] Abh. d. Sächs. Ges. d. Wiss. xvi, 185. 1890.

[6] Investigations on Protoplasm, 227. 1894. (German text 1892.)



WOODCUT 11 (Fig. 6).

Changes observed in the distal part of a free hypha during an hour and a half. Drops of water successively appeared on the surface, and through some of them branches eventually protruded: *a*, appearance at 4 p.m.; *b*, at 4.20; *c*, at 4.35; *d*, at 4.50; *e*, at 5; *f*, at 5.30.

in the same direction as on the previous day was seen, with occasional interruptions, and one backward movement for a distance of about twice the diameter of the filament. At 3.30 p.m. the observations were renewed, and for half an hour streaming in the same distal direction with occasional periods of quiescence was noted. At 4 o'clock a reverse movement of full strength continued for several minutes, then changed to the former distal course. In this way the reverse or proximal movement alternated several times with the distal movement during the next half hour. The observations were now abruptly terminated by an unfortunate displacement of the microscope.

It is possible that the drops upon the hyphae, which appear to precede streaming, are formed by the action of some excreted osmotic substance, as suggested by Wilson[1] in case of *Pilobolus crystallinus*; but it seems to me more probable that the position of the drops is determined by the localization of a cytohydrolytic ferment, which acting upon the wall renders it more permeable, and the internal pressure then forces out the water. This hypothesis would also explain the extension of this particular watery part of the cell-wall into a branch. A similar suggestion has been made by Marshall Ward[2] in connexion with the study of a species of *Botrytis*, in which the extrusion of enzym-drops was observed. But the drops upon the Mucoraceae are undoubtedly of a different nature from those seen by Marshall Ward, for in *Botrytis* they appear usually at the tips of the hyphae[3] and remain there as growth continues; in the Mucoraceae they are lateral, and the branches grow through and beyond them. An enzym has already been separated, in fact, from *Rhizopus nigricans* by Kean[4], which is capable of softening cellulose walls, and probably is produced by other members of the Mucoraceae; but in what part of the fungus it resides has not been determined.

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[1] Unters. a. d. bot. Inst. zu Tübingen, i, 15. 1881.

[2] Ann. Bot. ii, 331. 1888.

[3] l. c. 339.

[4] Bot. Gaz. xv, 173. 1890.

It is probable, if we consider the facts now in our possession, that in order for streaming to take place the filaments must have a high internal pressure. It is doubtless the pressure which forces water through places of less resistance in the walls, although the walls throughout are highly permeable, as shown by the rapidity with which the mycelium collapses upon removal from a moist to a dry atmosphere. The ready permeability of the walls will account for Wilson's results in obtaining drops by placing particles of sugar on the surface of *Pilobolus*. Internal pressure, as we know, is secured through osmotic action, and altogether it seems probable that the necessity for a very moist atmosphere to bring about streaming lies in the fact that the part of the mycelium in contact with the moist substratum cannot take up water fast enough to secure pressure throughout the whole structure so long as extravasated moisture is rapidly removed from the aerial surface of the very permeable hyphal walls.

By admitting the existence of a high osmotic pressure, we shall be able to explain many of the phenomena connected with the streaming. In the first place we must premise that although osmotic pressure originates in the movement of the molecules of the osmotic substance and varies directly as the density of the solution, yet it is converted into, and is manifested as, hydrostatic pressure which is uniformly distributed per unit area throughout the enclosing wall. The more tensely the cell-wall is stretched by the internal pressure, the more sensitive every part becomes to any variation in the pressure. If we imagine a hyphal tube some millimetres in length, under strong pressure, with one end in a watery substratum and the other in air, it is easy to see that any water taken up osmotically at the submerged end must instantly expand some part of the tube (growth), or force water out through the walls of the aerial part (extravasation). The water taken in at one end and discharged at the other displaces the whole column and moves it along toward the end where the water is escaping. But in the hyphal tube the water is an interrupted column lying in the more viscous protoplasm, and the movement will therefore be noticeably greater along the axis of the tube where there is least friction. For this reason every transverse surface tends to become more and more convex in the direction of the movement, which accounts for the heightened convexity of the forward end of the vacuoles and the flattening or introversion of the rear end.

There is, furthermore, less resistance to axial movement just in proportion to the thinness of the transverse layers of protoplasm ; therefore a thin-walled vacuole will tend to move faster than a thick-walled one; and this action, coupled with the tendency of the transverse layers to become thinner as movement progresses, dependent upon density, viscosity, elasticity and other factors, brings about the frothy appearance in the rear of a long vacuole.

It is evident that if the movement is due to the change in water-content at the extremities, streaming toward the free aerial parts should be more frequently seen than the opposite movement; and this is the case, the observation cited in detail being almost typical. It would also follow that streaming would be more constant toward and into growing branches and developing sporangia than toward other parts, for at these places both metabolism and extension of the walls are demanding material and removing resistance from the advancing column. This also agrees with observation, and in fact is the particular case noticed by De Vries, mentioned at the outset. The same greater proportion of movement toward advancing extremities and into sporangia has been observed by De Bary[1] in the streaming of the Myxogastres.

The number of conditions which may have an influence upon the movement is considerable, and their interaction very complex. But foremost among them, after sufficient turgidity is attained, is undoubtedly the facility of absorbing water in one part and its release in a distant part. Some attention was given to cases where this was minimized, and a specific instance or two may be cited. A culture of *Mucor racemosus*,

[1] Morph. and Biol. of Fungi and Myc., 426.

a species much given to the early formation of cross-walls in the hyphae, was grown upon neutral nutritive gelatin. This species branches acropetally in a most uniform manner, the branches making an angle of about sixty degrees with the main axis, and both growing quite straight. The advancing hyphae all remained appressed to the moist substratum, giving a regular featherlike appearance. On the second day movement was to be seen. At this time the central part of the mycelium bore plenty of sporangiophores, and was furthermore much septated. The protoplasm was crowded with vacuoles, but quite free from granules. The movement was not especially lively; in all cases it proceeded toward the growing apex, and although the whole branch-system was in normal growing condition, the stream mostly kept to the main axial hypha, only now and then entering a side branch. On the day following the movement still continued in the same direction. In two cases I was able to trace the stream back from the growing apex into the central mass of mycelium until a cross-wall was reached where all movement ceased. In these cases the whole length of the effective hypha was in contact with the moist substratum, although the proximal end was overlaid with growing mycelium, while the distal end, into which the stream poured, was both extending and half exposed to the air. I assume that in this case the small difference in osmotic conditions between the two ends of the active hyphae was the cause of the sluggishness of the current.

In another case still more uniform osmotic conditions were secured. Spores of *Rhizopus elegans* were grown in bread-broth entirely submerged. On the fifth day neither vacuoles nor movement were yet to be seen. On the tenth day vacuoles were abundant, and two or three instances of faint streaming were detected, but the development was no longer entirely beneath the surface, for a few sporangiophores had risen into the air.

In the last case another phenomenon of protoplasmic movement appeared, which had also been seen a number of times under less favourable conditions. The microsomes were in quite active locomotion through the protoplasm, exhibiting a kind of indefinite circulation. Far finer examples of this movement were obtained, however, in sowings of *Pilobolus* spores upon the surface of bread-broth. In twelve hours after sowing (overnight), they had germinated freely with long branching hyphae. Each spore contained a large vacuole, and a few small ones appeared in the hyphae. The microsomes were especially distinct, and kept up a lively coursing in all directions, but more especially lengthwise the hyphae and into and out of the spores. They appeared to follow no defined routes, which seems to indicate that they were not dependent upon circulating currents in the liquid. Moreover, sometimes collisions occurred between microsomes moving in opposite directions. I have no explanation of this phenomenon to offer; but do not think that it is connected in any direct way with the streaming movements in hyphae. It was necessary, however, to mention it, in order to distinguish it from the floating of microsomes in currents, which I now desire to describe.

I have so far said nothing about return currents in the hyphae, but they are occasionally to be seen. When the return current exists it is generally well defined, and always occupies the periphery of the hyphal cavity. It carries no vacuoles, and can only be detected by the movement of the microsomes. As the hypha is seen in optical section, there appears to be the usual surging, vacuolated stream moving through the centre, and on either side next to the walls a narrow uniform layer of clear protoplasm, in which minute particles are moving in opposite direction to the central stream. Between the two streams is a quiescent partition of protoplasm of about the same thickness as that lining the cell-walls. These quiescent layers vary much in thickness, and are at times so thin as to be scarcely discernible. It is obvious that whenever such return currents exist, their volume is ample to bring back all the protoplasm borne forward by the central stream, especially as it has become freed from all vacuolar sap, which has probably been used in growth or extravasated (Fig. 5).

It has been impossible in this presentation of my studies to touch upon a number of questions which have arisen during their progress, or to discuss fully those brought forward. I have attempted to give as briefly as possible the main facts regarding the streaming movement of the contents of hyphae where the coenocytic structure furnishes an uninterrupted passage of considerable length. The passage is usually some millimetres, and often many centimetres, in length, and often equals one-fourth millimetre, or even one-half millimetre, in diameter. I have brought together a number of observations which seem to me to clearly indicate that the movement is in the main a physical one, dependent upon osmotic conditions; although there can be little question that lying back of the physical factors the living protoplasm functions as a strong inciting and controlling agent. I have found, in fact, although not before mentioned, that streaming can be set up in a purely artificial manner that does not differ in any observable particular from the natural streaming. In a culture of *Rhizopus nigricans*, the application of a drop of a 20 per cent. solution of potassium nitrate caused a vigorous movement for a time through the hyphae toward the place of application. It is evident that this plasmolytic agent extracted the water from the filaments, thus causing the flow. After a time normal conditions prevailed in the hyphae experimented with. A 15 per cent. solution produced but slight movement. I need not recapitulate the conditions which, under normal relations, effect the plasmolytic changes.

I have observed the streaming movement in the following eight species of Mucoraceae: *Mucor Mucedo*, L.; *M. racemosus*, Fries; *Rhizopus nigricans*, Ehr.; *R. elegans* (Eidam), Ber. and De T.; *Phycomyces nitens* (Ag.), Kze.; *Sporodinia Aspergillus* (Scop), Schröt.; *Thamnidium elegans*, Lk.; and *Pilobolus crystallinus* (Wigg.), Tode. I do not doubt but that it occurs normally in many other coenocytic forms, where the conditions are favourable.

In conclusion I may express the opinion that the movement is an incidental feature in the life of the plant. It does, without doubt, aid in the transfer of nutrient material to points where growth is taking place, as De Vries has pointed out; but I believe that growth is not dependent upon it, and that full and normal development takes place without the movement coming into action.

The Correlation of Growth under the Influence of Injuries.

BY

C. O. TOWNSEND.

IT has been the purpose of the experiments which form the basis of this paper to determine in what time, through what distance, and to what extent an injury inflicted upon one part of a plant will influence the growth of the injured and of the uninjured parts. The experiments have been carried on in the Botanical Institute at Leipzig during the years 1896–7, under the direction of Professor Dr. Pfeffer, to whom I wish to express my sincere thanks for his invaluable suggestions, and also for the use of his splendidly equipped laboratory.

The correlation of growth has received considerable attention from investigators either directly or indirectly, but the observations have been made for the most part under the influence either of long-continued irritation or of a series of irritations produced at more or less irregular intervals, and extending over a considerable period of time[1]. In the following experiments special attention has been given to first effects of injury upon the curve of growth,

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[1] Pfeffer, Druck und Arbeitsleistung, 1893. Hering, Ueber Wachsthumscorrelation in Folge mechanischer Hemmung des Wachsens; Pringsheim's Jahrbuch, Vol. 29, p. 132, 1896. Kny, On Correlation in the Growth of Roots and Shoots; Annals of Botany, Vol. viii, p. 265, 1894.

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while previous investigators have concerned themselves for the most part with final results.

It is well known that the removal of some of the branches of a tree will often cause the other branches to become more vigorous and the fruit to be more perfectly developed; also that the disturbance of the roots of garden-plants, through the process of cultivation, often causes a more vigorous and rapid development of all their parts. However, no systematic study of injured plants has heretofore been made, in regard either to the effect of a single irritation of short duration or to the time required for such effect to manifest itself.

MATERIAL AND METHODS.

As far as possible in the experiments herein described, the external causes of the variation of the rate of growth were eliminated in order to study more accurately the internal influence of the irritation. With this object in view the plants were grown in damp chambers, in diffused light, and in all possible cases at a constant temperature.

The larger number of the experiments were performed with seedlings. Satisfactory results were obtained with seedlings of *Phaseolus multiflorus*, *Vicia Faba*, *Lupinus albus*, *Helianthus annuus*, *Cucurbita Pepo*, *Zea Mais*, *Avena sativa*, *Hordeum vulgare*, and *Secale cereale*. Experiments with older plants were carried on with the same material, also with *Calla*, and with a few cuttings of *Salix*. A number of experiments have also been performed with *Phycomyces nitens*.

Seedlings.—The seeds were soaked for about twenty-four hours, and then placed in damp sawdust at the temperature under which the experiments were to be carried on[1]. When the roots and shoots had attained the desired length, the seedlings were placed in glass boxes containing damp sawdust, or the seeds were wrapped in damp cotton, and the roots were suspended in water or in damp chambers. In order to study the growth of the roots after the shoots were injured, the roots were marked with indian ink about 15 mm. from the tip (distance accurately measured) before the seedlings were placed in the glass boxes. They were then left for several hours in order to determine the normal rate of growth. The plumule or shoot was afterwards removed from the odd numbers, by means of a sharp knife or a pair of scissors, leaving the even numbers in a normal state for control-plants. In other experiments seedlings were prepared as above, but the shoots were split for several millimeters instead of being removed. If it became necessary to remove one of the cotyledons in order to treat the plumule as desired, one cotyledon was also removed from each of the control-plants.

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[1] True, On the Influence of Sudden Changes of Turgor and of Temperature on Growth; Annals of Botany, Vol. ix, p. 363, 1895.

In studying the growth of the shoot when the root was injured, the seedlings were prepared as before, and the shoot was marked near the base. In some cases only the root-tips were removed; in other experiments nearly the whole root was cut away; while in still other experiments the roots were split, either near the base or near the tip, for a distance of ten millimeters. Cultures in which the roots were grown in water or in damp chambers were especially convenient in all experiments in which the roots were to be treated, and in the study of the growth of secondary roots.

To determine the effect upon the growth of the leaves when the tips of the leaves themselves were injured seedlings of *Avena*, *Hordeum*, and *Mais* were grown in damp sawdust, and, after determining the rate of growth for a given number of hours, the tips of the leaves were cut off from two to fifteen millimeters. In like manner the effect was observed upon the growth of stems when an entire leaf was removed from the stems of *Phaseolus*, *Vicia* and *Cucurbita*.

A series of experiments were carried on to determine the effect of an atmosphere of ether upon growth. For this purpose *Hordeum*, *Avena*, and *Secale* were found convenient. The seedlings were grown in small pots of damp sawdust under large bell-jars. After the normal rate of growth had been determined, a shallow dish containing 200 cc. of water, in which a definite amount of ether had been dissolved, was introduced under one of the bell-jars, the other preparation being left in a normal state for control. In some of the experiments the seedlings were kept in a dilute atmosphere of ether as long as they continued to grow, while in other cases they were placed in a strong atmosphere of ether for a short time. In order to measure the leaves, it was necessary to remove the plants from the bell-jars from time to time; and, as this allowed more or less of the ether to escape, a fresh mixture of ether and water was introduced under those bell-jars in which it was desired to maintain a constant atmosphere of ether.

Older Plants.—In preparing the older plants, seedlings were first started in damp sawdust, and then transferred to pots of damp earth and kept for several weeks under bell-jars. In order to have the root-tip in view, the main root was directed through the hole in the bottom of the flower-pot and allowed to extend into a damp chamber.

To study the growth of the root after the stem was injured, the root was marked 15 mm. from the tip, and the normal rate of growth observed. Only a part of the stem and a few of the leaves were then removed, and the growth of the root noted from time to time.

In considering the influence upon the stem when the roots were injured, a knife-blade 5½ cm. in length was passed around the main root at a distance of only a few centimeters, thus severing a larger or a smaller number of the secondary roots. In order to determine accurately the degree of injury which was inflicted by removing the roots, and also to eliminate the influence of transpiration, seedlings were placed in cylinders of plaster of Paris from three to four centimeters in diameter, the bottoms of which were composed of cotton-netting. The netting prevented the earth from falling through, but allowed the roots, which were directed into damp chambers, to pass freely. By this means a definite number and desired length of roots could be removed. *Calla* plants which had been grown for several months in pots of earth, were studied with reference to the growth of young leaves and roots when a full-grown vigorous leaf was removed. A few cuttings of *Salix* were prepared according to the method used by Kny[1].

Phycomyces.—A drop of gelatine containing the proper nourishing material was placed on a glass slide, and on the gelatine was deposited a single spore of *Phycomyces*. The preparations were kept in damp chambers in the dark until one or more sporangium-stalks formed. The damp chambers for these experiments consisted of air-tight boxes open at one end and two or three centimeters square by five or six centimeters long. These were lined on two opposite sides with moist filter-paper, and placed over the drop of gelatine on which the spore rested. By this means the growth of the sporangium-stalk could be observed in the damp chamber with the aid of a horizontal microscope. Having determined the rate of growth of the sporangium-stalk for a given interval, the damp chamber was removed for a moment, and the plant subjected to the following treatment:—

1. One or more of the mycelia were cut at a distance of from one to two millimeters from the base of the sporangium-stalk under investigation,

2. One of two or more sporangium-stalks produced by the same plant was cut off;

3. The mycelia were treated with a solution of potassium nitrate of definite strength.

In the experiments in which potassium nitrate was used, the volume of the gelatine-drop was liable to increase, causing an apparent elongation of the sporangium-stalk; hence in these experiments the sporangium-stalks were marked with indian ink below the growing zone[2].

In all higher plants the growth was measured at intervals of three, six, twelve, or twenty-four hours by means of a scale divided into half millimeters. For shorter intervals the growth of the higher plants, as well as of *Phycomyces*, was measured by means of a horizontal microscope. Control-plants were used in all cases except with *Phycomyces*, and were selected according to their equality in size and rate of growth with those to be treated.

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[1] L. c., p. 426.

[2] Études sur la Turgescence chez le Phycomyces, p. 22, 1885.

EXPERIMENTS.

Seedlings.—Owing to the high degree of independence existing between roots and shoots, it was necessary to perform a large number of experiments in order to reach definite and satisfactory conclusions. No attempt will be made to give a detailed description of the individual experiments, but rather the general results obtained. In the following tables a series was made up from the averages of growth of a large number of seedlings for a definite period. In the second column *P.* indicates the period during which the injured and the uninjured plants were compared. The periods were often determined from a large number of observations made at short intervals and combined; e.g. if four observations were made at intervals of six hours, and each observation showed an acceleration of growth, the four observations were combined and given as one observation of twenty-four hours. All the numbers opposite *a* were made up from the averages of nine or more experiment-plants; while the numbers opposite *b* were made up in the same manner from the same number of control-plants. The original length of roots and shoots indicates the length of these parts when measured for the determination of the normal rate of growth. The normal rate of growth was determined for all experiment as well as control-plants. The column headed 'Average growth during first period after injury' usually covered the time within which a change in rate of growth became apparent. The last column in each table is given for the purpose of showing how long the plants were under observation after treatment, and also for the purpose of comparing the growth of the injured and of the control-plants for the total period after injury. The difference between the increase of the injured and of the control-plants, however, does not indicate the actual acceleration or retardation of growth if the several short periods show both acceleration and retardation.

TABLE I.

Growth of root after shoot was injured.

Series I. *Zea Mais.* { *a.*—Entire shoot removed.
{ *b.*—Control.

Series II. *Vicia Faba.* { *a.*—Entire shoot removed.
{ *b.*—Control.

Series III. *Vicia Faba.* { *a.*—Plumule removed.
{ *b.*—Control.

Temperature, 22° (constant).

Series.		Average original length of root of twelve seedlings.	Average original length of shoot of twelve seedlings.	Average normal rate of growth of root.	Average growth of root, 1st period after injury.	Average growth of root, 2nd period after injury.	Average growth of root, 3rd period after injury.	Average total growth of root after injury.
I.	P.			24 hrs.	24 hrs.	48 hrs.	48 hrs.	120 hrs.
	a.	21 mm.	9·5 mm.	38 mm.	43 mm.	83 mm.	66 mm.	192 mm.
	b.	21 ,,	9 ,,	39 ,,	36 ,,	68 ,,	47 ,,	151 ,,
II.	P.			24 hrs.	24 hrs.	48 hrs.	144 hrs.	216 hrs.
	a.	50 mm.	14 mm.	14 mm.	21 mm.	36 mm.	124 mm.	181 mm.
	b.	50 ,,	16 ,,	14 ,,	27 ,,	36 ,,	72 ,,	135 ,,
III.	P.			24 hrs.	24 hrs.	24 hrs.	48 hrs.	96 hrs.
	a.	34 mm.		33 mm.	43 mm.	42 mm.	68 mm.	153 mm.
	b.	36 ,,		32 ,,	42 ,,	38 ,,	69 ,,	149 ,,

If we examine Table I, Series I and II, we shall find that the injury produced an acceleration in the growth of the root as a final result in both series. In Series I the acceleration began within twenty-four hours, and continued for five days. In Series II a retardation was observed during the first twenty-four hours, followed by a period of normal growth, which in turn was followed by a period of acceleration. Series III, Table I, shows neither acceleration nor retardation.

If we now turn to Table II, it will be seen that the shoot was influenced by an injury produced upon the root. The final result of Series I, Table II, would indicate that no change had taken place in the rate of growth, but an examination of the figures under the first period after injury shows a distinct retardation, while the figures under the third period indicate a slightly stronger acceleration. In Series II, Table I, the retardation became apparent within twenty-four hours, and continued for three days, followed by a period of acceleration which also lasted for three days. Series III of the

TABLE II.

Growth of leaf after root was injured.

Series I. *Hordeum.* *a.*—5 mm. removed from root-tip.
b.—Control.
Series II. *Zea Mais.* *a.*—Three-fourths of each root removed.
b.—Control.
Series III. *Zea Mais.* *a.*—Entire root removed.
b.—Control.

Temperature, 22° (constant).

Series.		Average original length of root of twelve seedlings.	Average original length of shoot of twelve seedlings.	Average normal rate of growth of leaf.	Average growth of leaf, 1st period after injury.	Average growth of leaf, 2nd period after injury.	Average growth of leaf, 3rd period after injury.	Average total growth of leaf after injury.
I.	P.			24 hrs.	24 hrs.	24 hrs.	24 hrs.	72 hrs.
	a.	28 mm.	8 mm.	20 mm.	30 mm.	37 mm.	31 mm.	98 mm.
	b.	29 ,,	8 ,,	19 ,,	38 ,,	40 ,,	19 ,,	97 ,,
II.	P.			24 hrs.	24 hrs.	48 hrs.	96 hrs.	168 hrs.
	a.	48 mm.	29 mm.	26 mm.	24 mm.	57 mm.	123 mm.	204 mm.
	b.	48 ,,	26 ,,	25 ,,	29 ,,	70 ,,	88 ,,	187 ,,
III.	P.			24 hrs.	24 hrs.	48 hrs.	48 hrs.	120 hrs.
	a.	57 mm.	23 mm.	16 mm.	17 mm.	31 mm.	19 mm.	67 mm.
	b.	56 ,,	21 ,,	15 ,,	20 ,,	39 ,,	61 ,,	120 ,,

TABLE III.

Growth of shoot after root was injured.

Series I. *Helianthus.*
a.—10 mm. removed from root-tip.
b.—Entire root removed.
c.—Control.

Temperature, 22° (constant).

Series.		Average original length of root of nine seedlings.	Average original length of shoot of nine seedlings.	Average normal rate of growth of shoot.	Average growth of shoot, 1st period after injury.	Average growth of shoot, 2nd period after injury.	Average growth of shoot, 3rd period after injury.	Average total growth of shoot after injury.
I.	P.			24 hrs.	24 hrs.	48 hrs.	144 hrs.	216 hrs.
	a.	66 mm.	22 mm.	10 mm.	17 mm.	65 mm.	92 mm.	174 mm.
	b.	64 „	21 „	10 „	13 „	35 „	41 „	89 „
	c	62 „	22 „	9 „	20 „	50 „	60 „	130 „

TABLE IV.

Growth of root and shoot after root-tip was slightly injured.

Series I. *Vicia Faba.*
a.—1 mm. (as nearly as possible) removed from root-tip.
b.—Control.

Temperature, 22° (constant).

Series.		Average original length of root of ten seedlings.	Average original length of shoot of ten seedlings.	Average normal rate of growth of root.	Average growth of root, 1st period after injury.	Average growth of root, 2nd period after injury.	Average growth of root, 3rd period after injury.	Average total growth of root after injury.	Average total growth of shoot after injury.
I.	P.			24 hrs.	24 hrs.	48 hrs.	144 hrs.	216 hrs.	216 hrs.
	a.	20 mm.	3 mm.	15 mm.	18 mm.	22 mm.	41 mm.	81 mm.	53 mm.
	b.	19 „	3 „	14 „	18 „	32 „	78 „	128 „	32 „

same table shows a constant retardation, although the retardation during the first period of twenty-four hours was no more marked than in Series I and II, in which the degree of injury was much less.

Table III shows the same general results with *Helianthus* as those shown in Table II with *Hordeum* and *Mais*. The periods of variation in the rate of growth, however, were longer in the experiments with *Helianthus*.

TABLE V.

Growth of leaf after leaf-tip was injured.

Series I. *Avena.*
a.—10 mm. removed from leaf-tip.
b.—5 " " "
c.—Control.

Series II. *Zea Mais.*
a.—5 mm. removed from leaf-tip.
b.—2 " " "
c.—Control.

Temperature, 22° (constant).

* Series.		Average original length of leaf of ten seedlings.	Average normal rate of growth of leaf.	Average growth of leaf, 1st period after injury.	Average growth of leaf, 2nd period after injury.	Average growth of leaf, 3rd period after injury.	Average total growth of leaf after injury.
I.	P.		24 hrs.	24 hrs.	48 hrs.	72 hrs.	144 hrs.
	a.	36 mm.	12 mm.	12 mm.	61 mm.	54 mm.	127 mm.
	b.	35 "	13 "	13 "	61 "	63 "	137 "
	c.	36 "	13 "	13 "	58 "	33 "	104 "
II.	P.		24 hrs.	24 hrs.	48 hrs.	168 hrs.	240 hrs.
	a.	18 mm.	10 mm.	11 mm.	25 mm.	93 mm.	129 mm.
	b.	20 "	9 "	11 "	33 "	135 "	179 "
	c.	19 "	10 "	11 "	29 "	71 "	111 "

According to Table IV even one millimeter cut from root-tips produced an acceleration in the growth of the shoots, although the growth of the root was of course retarded. During the first twenty-four hours after cutting no change could be detected in the rate of growth of either root or shoot, but during the following nine days the rate of growth of the roots was distinctly retarded, while the rate of growth of the shoots was just as distinctly accelerated, though not to the same extent.

When leaf-tips were cut off as in Table V, the growth of the leaves cut was accelerated if the growing zone was not injured. In Series I, Table V, the acceleration began after the first twenty-four hours after injury, and continued for five days, i. e. during the whole growth of these leaves of the seedlings.

TABLE VI.

Growth of shoot after one leaf was removed.

Series I. { *a.*—One leaf removed.
Phaseolus. { *b.*—Control.

Temperature, 23° (constant).

Series.		Average original length of shoot of twelve seedlings.	Average normal rate of growth of shoot.	Average growth of shoot, 1st period after injury.	Average growth of shoot, 2nd period after injury.	Average growth of shoot, 3rd period after injury.	Average total growth of shoot after injury.
I.	P.		24 hrs.	24 hrs.	96 hrs.	192 hrs.	312 hrs.
	a.	164 mm.	9 mm.	22 mm.	104 mm.	66 mm.	192 mm.
	b	162 ,,	9 ,,	17 ,,	63 ,,	31 ,,	111 ,,

In Series II *a* shows a slight retardation after the first twenty-four hours followed by acceleration, while *b* shows continuous acceleration after the first twenty-four hours, returning to a normal growth after about nine days.

The effect of removing one of the leaves from young shoots was next investigated. For this purpose *Phaseolus multiflorus* was found to give very satisfactory results. Table VI shows that the acceleration in growth began within twenty-four hours after injury, and continued for about thirteen days, showing a total acceleration of more than 70 °/o of the normal growth.

In Table VII, in which the shoots were split along one side, a retardation in the growth of the shoots was appreciable during the first twenty-four hours. This was followed by a period of acceleration which continued for five days, at the end of which time the growth-rate had again become normal.

When the roots were split, no variation was noticeable in either root or leaf during the first period of twenty-four hours; but after the first twenty-four hours acceleration began, and continued for five days in both root and leaf, as shown in the last two columns in Table VIII. The secondary roots were longer and more numerous in the injured than in the uninjured plants[1].

TABLE VII.

Growth of shoot after the shoot was split on one side.

Series I. *Heliainthus.* { *a.*—Shoot split 10 mm. on one side near the base.
{ *b.*—Control.

Temperature, 22° (constant).

Series.		Average original length of shoot of nine seedlings.	Average normal rate of growth of shoot.	Average growth of shoot, 1st period after injury.	Average growth of shoot, 2nd period after injury.	Average growth of shoot, 3rd period after injury.	Average growth of shoot, 4th period after injury.	Average total growth of shoot after injury.
I.	P.		24 hrs.	24 hrs.	48 hrs.	96 hrs.	96 hrs.	264 hrs.
	a.	29 mm.	13 mm.	15 mm.	35 mm.	27 mm.	19 mm.	96 mm.
	b.	30 ,,	12 ,,	22 ,,	26 ,,	18 ,,	18 ,,	84 ,,

A closer study of secondary roots showed that their growth was first retarded and afterwards strongly accelerated under the influence of injury as seen in Table IX. Experiment *b* shows that the splitting of the roots caused acceleration in the growth of shoots as well as of main and secondary roots. The number of secondary roots was also increased if too much of the main root had not been removed, or if the main root was split (*b*, Table IX).

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[1] Pfeffer, Druck und Arbeitsleistung, p. 343, 1893.

TABLE VIII.

Growth of leaf and root after the root was split near the tip.

Series I. *Zea Mais.* { *a.*—Roots split 10 mm., beginning 5 mm. from the tip.
b.—Control.

Temperature, 22° (constant).

Series.		Average original length of root of twelve seedlings.	Average original length of shoot of twelve seedlings.	Average normal rate of growth of leaf.	Average growth of leaf, 1st period after injury.	Average growth of leaf, 2nd period after injury.	Average growth of leaf, 3rd period after injury.	Average total growth of leaf after injury.	Average total growth of root after injury.
I.	P.			24 hrs.	24 hrs.	48 hrs.	72 hrs.	168 hrs.	168 hrs.
	a.	26 mm.	10 mm.	15 mm.	15 mm.	55 mm.	78 mm.	148 mm.	166 mm.
	b.	25 "	11 "	15 "	16 "	43 "	62 "	121 "	134 "

TABLE IX.

Growth of roots and shoots after the roots were injured.

Series I. *Phaseolus multiflorus.* { *a.*—10 mm. removed from root-tips.
b.—Roots split 10 mm., beginning 5 mm. from the tip.
c.—Control.

Temperature, 22° (constant).

Series.		Average original length of root of eleven seedlings.	Average original length of shoot of eleven seedlings.	Average length of secondary roots at end of 1st period.	Average length of secondary roots at end of 2nd period.	Average length of secondary roots at end of 3rd period.	Number of secondary roots at end of experiment.	Average total length of main root.	Average total length of shoot after injury.
I.	P.			24 hrs.	24 hrs.	240 hrs.	288 hrs.	288 hrs.	288 hrs.
	a.	38 mm.	17 mm.	68 mm.	260 mm.	3100 mm.	26	28 mm.	225 mm.
	b.	37 "	18 "	53 "	210 "	3300 "	42	215 "	213 "
	c.	35 "	17 "	82 "	210 "	2100 "	34	180 "	176 "

When the plants were kept in a constant atmosphere of ether derived from 1 cc. of ether in 200 cc. of water (Series I, Table X), a constant retardation was observed, although the plants remained alive and grew for the same length of time as the control-plants, viz. about six days. If a stronger atmosphere than the above was used, a still more marked retardation was apparent. On the other hand, if a weaker atmosphere of ether was used, e. g. $\frac{1}{10}$ cc. of ether in 200 cc. of water (Series II, Table X), the growth was first retarded and then distinctly accelerated, the younger plants being more easily retarded in their growth than were the older ones. If less than $\frac{1}{10}$ cc. of ether was used, no change which could not have been due to individual differences could be detected.

TABLE X.

Growth of leaves in a constant atmosphere of ether.

Series I. *Avena.* { *a.*—1 cc. of ether in 200 cc. of water (under bell-jars).
b.—Control.

Series II. *Avena.* { *a.*—$\frac{1}{10}$ cc. of ether in 200 cc. of water (under bell-jars).
b.—Control.

Temperature, 20° (constant).

Series.		Average original length of leaves of twelve seedlings.	Average normal rate of growth of leaves.	Average growth of leaves, 1st period in ether.	Average growth of leaves, 2nd period in ether.	Average growth of leaves, 3rd period in ether.	Average growth of leaves, 4th period in ether.	Average total growth of leaves in ether.
I.	P.		24 hrs.	24 hrs.	24 hrs.	48 hrs.	48 hrs.	144 hrs.
	a.	39 mm.	16 mm.	21 mm.	25 mm.	38 mm.	12 mm.	96 mm.
	b.	39 „	16 „	27 „	34 „	56 „	17 „	134 „
II.	P.		24 hrs.	24 hrs.	48 hrs.	48 hrs.	72 hrs.	192 hrs.
	a.	26 mm.	1 mm.	21 mm.	66 mm.	40 mm.	34 mm.	161 mm.
	b.	26 „	11 „	28 „	58 „	31 „	17 „	134 „

Series I, Table XI, shows that seedlings may be shocked for one hour with strong ether without producing any marked effect upon the rate of growth of the leaves. If the shock lasted one and a half hours, as in Series II, an acceleration was noticeable, beginning after the first twenty-four hours and continuing four days, i. e. as long as the leaves of the seedlings grew. If the shock was allowed to continue for two hours, a marked retardation during the first forty-eight hours was observed, followed by a return to the normal rate of growth, but without acceleration (Series III). A shock

TABLE XI.

Growth of leaves after shocking with ether.

Series I. *Avena.* { *a.*—1 hour in atmosphere of ether (5 cc. ether in 200 cc. water). *b.*—Control.
Series II. *Avena.* { *a.*—1½ hours in atmosphere of ether (5 cc. ether in 200 cc. water). *b.*—Control.
Series III. *Avena.* { *a.*—2 hours in atmosphere of ether (5 cc. ether in 200 cc. water). *b.*—Control.

Temperature, 23° (constant).

Series.		Average original length of leaf of twelve seedlings.	Average normal rate of growth of leaves.	Average growth of leaves, 1st period after shocking.	Average growth of leaves, 2nd period after shocking.	Average growth of leaves, 3rd period after shocking.	Average growth of leaves, 4th period after shocking.	Average total growth of leaves after shocking.
	P.		24 hrs.	12 hrs.	12 hrs.	24 hrs.	72 hrs.	120 hrs.
I.	*a.*	25 mm.	21 mm.	14 mm.	14 mm.	22 mm.	29 mm.	79 mm.
	b.	26 „	22 „	17 „	15 „	25 „	25 „	82 „
	P.		24 hrs.	24 hrs.	24 hrs.	24 hrs.	48 hrs.	120 hrs.
II.	*a.*	33 mm.	27 mm.	30 mm.	30 mm.	25 mm.	11 mm.	96 mm.
	b.	35 „	25 „	30 „	25 „	16 „	2 „	73 „
	P.		24 hrs.	12 hrs.	36 hrs.	24 hrs.	48 hrs.	120 hrs.
III.	*a.*	26 mm.	29 mm.	15 mm.	32 mm.	20 mm.	7 mm.	74 mm.
	b.	28 „	28 „	21 „	46 „	22 „	7 „	96 „

of longer duration than two hours usually caused either a continuous retardation or death.

Older Plants. Stems.—Satisfactory results were obtained in studying the growth of the stems when the roots were injured in plants of *Phaseolus multiflorus* about two weeks old. In the experiments with these older plants a single root was allowed to pass through the bottom of the flower-pot in the manner already described. This root was then allowed to attain a length of from thirty to forty centimeters below the bottom of the flower-pot before cutting away, but no appreciable variation in the rate of growth of the stem could be detected. Subsequent experiments showed that the injury in this case was not strong enough to produce any decided change in the rate of growth. However, when a large number of the secondary roots were cut off at a distance of about two centimeters from the main root, as in Table XII, a decided acceleration in the growth of the stem took place within twelve hours after injury, and continued for about forty-eight hours. This was followed by a period of normal growth lasting about twenty-four hours, after which came a period of retardation lasting about five days, at the end of which time the stems were again practically equal in

TABLE XII.

Growth of stem after a number of secondary roots were removed.

Series I. *Phaseolus.* { *a.*—About two-thirds of the secondary roots removed 2 cm. from base.
{ *b.*—Control.

Temperature 22° (constant).

Series.		Average original length of stem of ten plants.	Average normal rate of growth of stems.	Average growth of stems, 1st period after injury.	Average growth of stems, 2nd period after injury.	Average growth of stems, 3rd period after injury.	Average growth of stems, 4th period after injury.	Average growth of stems, 5th period after injury.	Average total growth of stems after injury.
	P.		24 hrs.	12 hrs.	12 hrs.	24 hrs.	24 hrs.	120 hrs.	192 hrs.
I.	*a.*	149 mm.	27 mm.	17 mm.	19 mm.	41 mm.	33 mm.	149 mm.	259 mm.
	b.	147 „	26 „	12 „	13 „	29 „	31 „	172 „	257 „

length, and the normal rate of growth was resumed. It was possible to determine approximately the degree of injury which had been inflicted upon these plants when they were removed from the earth at the end of the experiments. When a number of the roots were forced to grow into the damp chambers and were then cut away, the results were similar to those given in Table XII, though in most cases not so marked, since the injury in the latter experiments was not so great, and since it was necessary for the influence to act through a greater distance. The number of roots cut off was only about one-third as many as in the previous experiments, and the irritation had to act through nearly twice the distance.

TABLE XIII.

Growth of a leaf after another leaf was removed.

Series I. *Calla*. { *a*, *b*, and *c*.—One leaf removed.
d.—Control.

Temperature, 17–21°.

Series.		Average original length of leaf of five plants.	Average normal rate of growth of leaves.	Average growth of leaves, 1st period after injury.	Average growth of leaves, 2nd period after injury.	Average growth of leaves, 3rd period after injury.	Average growth of leaves, 4th period after injury.	Average growth of leaves, 5th period after injury.	Average total growth of leaves, after injury.
I.	P.		24 hrs.	6 hrs.	24 hrs.	24 hrs.	24 hrs.	96 hrs.	174 hrs.
	a.	245 mm.	22 mm.	10 mm.	32 mm.	30 mm.	27 mm.	68 mm.	167 mm.
	b.	150 „	17 „	8 „	25 „	18 „	10 „	40 „	101 „
	c.	211 „	20 „	13 „	37 „	45 „	25 „	78 „	198 „
	d.	185 „	18 „	4·5 „	19 „	18 „	17 „	64 „	122·5 „

Leaves.—The growth of young leaves of *Calla* was found to be nearly uniform from day to day when the conditions were normal and constant. If however the plant was disturbed by the removal of one of the full-grown vigorous leaves, the rate of growth of the young leaves was decidedly accelerated within six hours. The acceleration continued from one to five days, after which came a period of normal growth, followed in some cases by a period of retardation (Table XIII).

Roots.—No appreciable change in the rate of growth of roots could be detected in the older plants, even when the entire stem, of *Phaseolus* for example, was cut away, leaving only the first pair of leaves. Also in *Calla* no change was produced in the rate of growth of the roots by the removal of a leaf, although this same injury produced such a comparatively sudden and marked change in the rate of growth of the young leaves.

Cuttings.—A few experiments were performed with cuttings of *Salix*, the results of which were similar to those already shown. Owing however to the small number of experiments and to the slight changes in the rate of growth, further investigations are necessary before the results can be tabulated with satisfaction.

Phycomyces.—When the mycelia were cut near the base of the sporangium-stalk, the rate of growth of the stalk was immediately reduced in a very marked degree, but in no case examined did the growth entirely cease. The sporangium-stalk began in a few minutes to recover its rate of growth, and in from thirty to sixty minutes was growing again at the usual rate. No change was observed in the rate of growth of the sporangium-stalk if the mycelia cut were comparatively thin, or if the larger mycelia were cut only near the tips. In those experiments in which one of two or more sporangium-stalks was cut off, the uninjured stalk showed a still stronger retardation in the rate of growth than when the mycelia were cut, and the retardation in some cases continued for a longer time. But, as in the preceding experiments, the growth did not absolutely cease.

While the above table would indicate that observations were made only at intervals of ten minutes, it was really the case that the plants were not removed from before the microscope. The treating and refocussing required but a few seconds, and the stalks were watched closely for the first few seconds after treating. After the rate of growth of a stalk had recovered from the effect of cutting one mycelium, the rate of growth was again reduced to the same extent if another mycelium of the same size was cut, the period of recovery being shorter usually in the second case

TABLE XIV.

Growth of sporangium-stalks of *Phycomyces* after other sporangium-stalks or mycelia were cut off or treated with KNO_3.

Temperature, 17–20°.

	Observation periods.	Growth of sp.-stalk after mycelia cut once.	Growth of sp.-stalk after mycelia cut twice.	Growth of sp.-stalk after another sp.-stalk was cut once.	Growth of sp.-stalk after another sp.-stalk was cut three times.	Helioptropism of sp.-stalk after mycelia were cut once.	Growth of sp.-stalk after KNO_3 applied to mycelia.
		Spaces.	Spaces.	Spaces.	Spaces.	Spaces.	Spaces.
N	10 min.	11	7	20	20	3	8
	"	12	7	20	22	3	8
	"	12*	8*	21*	23*	3*	9*
	"	3	3	1	1	½	5
	"	4	4	8	10	½	2
	"	5	6	15	14	1	0
	"	7	8*	20	18*	1½	0
	"	10	3	20	18	2	0†
	"	11	5	22	20*	3	0
	"	13	8	25	22	3	0
	"	14	9	26	22	3	1
	"	15	9	26	25	4	3

One space = $\frac{1}{30}$ of a millimeter.
N shows normal growth for three periods.
* Indicates the time of injury.
† In the last column indicates that the 5% KNO_3 solution was gradually displaced by water.

(Table XIV, column 3). If a stalk was cut, the reduction in growth was greater than when a mycelium was cut (Table XIV, column 4). In column 5, Table XIV, the same stalk was cut three times. The first time about one-third of the stalk was removed, causing a decided retardation in growth of another stalk on the same plant. The second and third cuttings removed the remaining two-thirds of the same stalk, but produced no change in the rate of growth of the stalk under observation.

When a solution of from 3 to 5% of KNO_3 was applied to the mycelia of *Phycomyces*, the growth of the stalk was gradually retarded until it finally ceased. If the turgor was restored by displacing the KNO_3 solution with water, the growth began again in from twenty to sixty minutes. The length of time required for the resumption of growth depended upon the strength of the KNO_3 solution used, and upon the time during which the growth had ceased. Cross-walls formed in the mycelia in from one to two hours after cutting, while the return to the normal rate of growth usually took place in from thirty to sixty minutes; hence it is clear that the formation of cross-walls could not have influenced the return to the normal rate of growth.

CONCLUSIONS.

Distance through which the irritation acted[1]. When nearly the entire root of seedlings was removed, the point of injury was only from one to ten millimeters from the lowest point of the growing-zone of the shoot. Likewise when the leaf-tips were removed (as in Table V), or a leaf was cut from the growing shoot (as in Table VI), the shortest distance to the zone of growth did not exceed a few millimeters. On the other hand, if the root-tip alone was removed or split, the irritation had to act through nearly the entire length of the root in order to affect the growth of the shoot. This distance, as shown by the tables, varied from twenty to fifty millimeters. In like manner, when the shoot was injured, it was necessary for the influence of the irritation to travel nearly the entire length of the root in order to affect the growing-zone. Furthermore it was not sufficient for the irritation simply to reach the growing-zone, but it was necessary for it to extend through a part or all of the cells of the zone in order to affect the growth.

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[1] Rothert, Ueber Heliotropismus; Cohn's Beiträge zur Biologie der Pflanzen, Vol. vii, p. 66, 1896.

In the older plants the distance through which the irritation acted was much greater than in the seedlings. In *Phaseolus*, for example, the entire growth of the stem, after the roots were injured, took place above the first pair of leaves, which were from 95 to 150 mm. above the root. The secondary roots were injured at least 20 mm. from the main root, so that the distance from the point of injury to the growing-zone of the stem was from 115 to 170 mm. In the *Phaseolus* plants in which the roots extended into damp chambers before injury, the distance was still greater. The flower-pots used were 110 mm. deep. This added to the 95 or 150 mm. made a distance of from 205 to 260 mm. through which the influence must have acted. In *Calla* the distance between the point of injury and the zone of growth varied from 50 to 80 mm. In *Phycomyces* the distance was from 10 to 50 mm.

These distances are by no means to be regarded as the limits through which such irritation will influence growth. The distance through which an influence will act depends upon the degree of injury and upon the external and internal conditions of the individual plants.

Time.—In experiments relative to the time required for the influence of the irritation to exhibit itself, observation with the horizontal microscope showed that there was no sudden change, due to injury, in the rate of growth of higher plants. It was only by extending the period of observation over several hours that a distinct difference in the rate of growth could be detected. It is therefore impossible to say definitely at what time an acceleration or a retardation of growth began.

In some of the experiments a distinct difference in the rate of growth was observed in six hours, while in other cases no change could be seen within twenty-four hours after injury. Even among plants of the same species and under the same conditions there were individual differences in the time required for the variation in the rate of growth. It was rare that a change in the rate of growth which could not be accounted for by individual differences was noticeable in less than six hours. The duration of the retardation or acceleration depended largely upon the degree of injury. If the injury was sufficiently severe, the retardation continued throughout the life of the seedling; while with a less severe injury the retardation lasted only from one to three days, and was often followed by a period of acceleration (Table II, Series II and III). If the injury was slight, it was usually followed by acceleration without an intervening period of retardation (Table VI). The influence of the irritation upon the seedlings was usually much more gradual than upon the older plants. The total period covered by a variation in the rate of growth due to a single irritation varied from one to twelve days, and depended upon the condition of the individual plant and upon the degree of injury. It was possible for the retarding and accelerating influences to neutralize each other so that the final result was the same as if no injury had been inflicted. Indeed it was possible for the final result to indicate a slower growth than the normal even though the injury was followed by a period of acceleration, provided that a strong retardation followed or preceded the acceleration.

With *Phycomyces* a reduction in the rate of growth took place immediately after cutting, as shown in Table XIV. The retardation was evidently due to a change of turgor rather than to an irritation produced by cutting. At any rate, it is impossible to know what part the irritation plays in these experiments so long as the influence of turgor is not eliminated.

Extent of variation in growth.—It is apparent from a study of the preceding tables that a change in the rate of growth due to injury may vary from 0 to 70 %. of the normal rate. It is evident that some plants respond much more readily to the influence of an irritation than others. This accounts in part for the great variation in different species. It appears however that an injury has two opposite effects upon growth, one of acceleration, the other of retardation. It is clear that these opposing tendencies may be so nearly equal that, although the plant is a sensitive one, no appreciable change in the rate of growth can be observed. If the tendency of the irritation to retard the growth is weaker or of shorter duration than is the tendency to acceleration, the plant will soon show signs of acceleration and *vice versa*. It is seen from the tables that the retarding influence is stronger than the accelerating influence under a severe injury, but that the retarding influence diminishes more rapidly than does the accelerating influence. It is impossible to determine if the retarding influence ever ceases entirely, or if the highest degree of acceleration is ever reached. We can only say that the amount of variation in growth is the difference between the accelerating and the retarding tendencies. If these opposing tendencies are in a state of equilibrium, the growth will be constant from period to period. The activities of growth[1] may serve as an irritant to disturb this state of equilibrium, as shown by the almost constant variation in the rate of growth of normal plants.

SUMMARY

A single irritation produced by cutting or splitting the shoots or roots or removing the leaf-tips of seedlings tends to produce a change in the rate of growth of the injured and of the uninjured parts.

If the injury is slight, signs of an acceleration in the rate of growth will be apparent in from six to twenty-four hours, and will continue for from one to several days. If the injury is severe, the acceleration will be preceded by a period of retardation of longer or shorter duration, depending upon the severity of the injury and upon the condition of the plant injured.

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[1] Pfeffer, Studien zur Energetik der Pflanze, 1892.

The growth of the stems of older plants is accelerated by removal of a number of the roots or leaves, but is not affected by a slight injury to the roots.

The roots of older plants as well as of seedlings are more independent than are the stems or shoots[1].

The change in the rate of growth of higher plants under the influence of a single irritation begins gradually, reaches its maximum in from twelve to ninety-six hours, and gradually diminishes until the normal rate is resumed.

A dilute but continuous atmosphere of ether, or a strong shock of ether of short duration, will produce an acceleration in growth.

The total variation in the growth of higher plants due to the influence of a single irritation is from 0 to 70°/₀ of the normal growth for the same period.

The growth of sporangium-stalks of *Phycomyces* is suddenly and strongly retarded by cutting either the mycelia or another sporangium-stalk on the same plant. The growth does not entirely cease, and gradually recovers its normal rate in from thirty to sixty minutes.

The influence of an irritation due to cutting or other injury is capable of acting through a distance of several hundred millimeters.

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[1] Kny, l.c., p. 280.

On the Structure of a Hybrid Fern.

BY

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With Plates XXIII and XXIV.

THE Fern which forms the subject of the present communication, *Polypodium Schneideri*, was first raised at Messrs. Veitch's, Chelsea, by Mr. Schneider several years ago, and he has several times succeeded in producing it afresh. The two parents were *P. aureum*, and a variety of *P. vulgare* known as *elegantissimum*. The latter plant occurs wild in some parts of Cornwall, and it shows some tendency to revert, in the form of its leaves, to the common *P. vulgare* type. The spores from the varietal leaf-form were sown, and when mature prothallia had been secured, they were planted along with prothallia from *P. aureum*. This method had to be adopted on account of the very different rates of development of the gametophyte in the two species respectively, that of *P. aureum* being relatively very rapid.

I may say that I have had a conversation on the subject of the Fern with Mr. Schneider himself, who kindly showed

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me his extensive collection of plants at the Chelsea Nurseries, and I have no doubt whatever as to the genuine hybrid nature of the Fern, both on the ground of what I saw there, as well as on account of the strong evidence in its favour furnished by the comparative study of its anatomy. The results of the successive fresh raising of the hybrid were strikingly uniform, the intermediate characters being prominent in all.

In the robust habit of growth and in its thick and densely ramentose rhizome, the hybrid approximates far more closely to the character of *P. aureum* than to that of *P. elegantissimum*[1], which possesses, relatively speaking, a slender rhizome which is not specially densely clothed with scales. Again, the presence of these ramenta (although but sparsely scattered) on the rachis of the young fronds of *P. aureum*, and, though in a less degree, on those of *P. Schneideri* also, appears in marked contrast with the naked leaf-stalk of the other parent. And this is the more singular inasmuch as in other respects the general appearance of the frond of the hybrid, save in its far larger size, very closely resembles that of *P. elegantissimum*, and is not at all like that of *P. aureum*.

It has been said that *P. elegantissimum* shows a tendency to 'revert[2]' to the simpler form of *P. vulgare*, and the same peculiarity is manifested in its bastard offspring. Sometimes only a few pinnae, or even a half-pinna, may exhibit the 'reversion' (Pl. XXIV, Figs. 17, 18); sometimes it extends to all the leaves on a branch (Fig. 19), and I saw a number of plants obtained from cuttings of such branches which had grown and remained true. But the greater number of reverted fronds of the hybrid were not quite like those of the *P. vulgare* type. Some of them exhibited the bluish bloom, and others the peculiarly wavy texture so characteristic of *P. aureum*. It was in the general outline that the

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[1] I use the curtailed form of the name for the sake of brevity.

[2] The use of the terms 'revert' and 'reversion' is merely one of convenience, and is not intended as implying an expression of opinion on the general questions of atavistic or discontinuous variations.

P. vulgare parentage was most strongly pronounced. The differences, *inter se*, of these reverted fronds is, I conceive, one of special interest, as affording a striking illustration of the unstable character of the hybrid.

Turning to the internal structure of the stem and of the petiole, we find a close approximation, though not a complete resemblance, between *P. Schneideri* and *P. aureum*.

The rhizome is almost exactly alike in the two cases, if one makes due allowance for a certain disparity of size. In both forms the vascular system is more complex, and the steles are larger than are those in *P. elegantissimum*.

In the leaf-stalk of *P. Schneideri* the preponderance of *aureum* characters is clearly marked, though in a less degree than in the rhizome. Thus an average-sized frond of *P. aureum* was found to possess, at the base of the stalk, two large steles beneath the adaxial face, together with four or five small ones, the whole group being disposed in a circle, as is shown in Pl. XXIII, Figs. 1, 2, 3. The number of the small steles varies irregularly at different heights of the petiole, owing to the frequent anastomoses and repeated divarications which occur amongst them. On the other hand, the course of the two large adaxial steles is very regular. They run in a nearly parallel direction up the leaf-stalk, only fusing at a short distance below the region of the lamina. As regards *P. elegantissimum*, the petiole possesses a far less complex vascular system. At the base, two large adaxial steles can be seen, accompanied usually by one but sometimes by two very much attenuated strands, corresponding to the four or five subsidiary steles of *P. aureum*. At a very short distance up the stalk, however, the two larger strands can alone be seen, and these fuse together about half-way up the petiole (Pl. XXIII, Fig. 5). An inspection of Figs. 1-8 on Plate XXIII will serve better than a description to illustrate the much greater approximation of *P. Schneideri* to *P. aureum*, as regards its vascular system, than to its other parent. The two large steles fuse rather earlier in the hybrid, and the number of the subsidiary is, on the average, rather less and their size is ller, than in *P. aureum*.

It is not a valid objection to the legitimacy of instituting these comparisons to urge that the degree of vascular complexity and development is merely conditioned by the size and luxuriance of the organ concerned. Of course these factors proximately determine whether large or small channels shall be sufficient, but they do not necessarily affect the rticular method of arrangement, or the relative proportions isting between the various constituent strands.

Turning to the sclerenchyma which occurs at the periphery the leaf-stalk, it is seen that in this particular also the
more closely res ne parent (*P. aureum*) than
other. The band is a little thicker, relatively
diameter of the section in *P. Schneideri*,
e cells are somewhat strongly lignified. In this
approach *P. elegantiss* in which the band is
p far more developed, its cells are
a are dovetailed each other more effec-
and are both more ed and lignified than in
eum.

As the leaf-blades of *P. Schneideri* and *P. elegantissimum* more nearly resemble each other, a considerable degree of similarity might be looked for in their epidermal cells and stomata. The resemblance as a matter of fact is fairly close (Pl. XXIII, Figs. 10, 11), but there are obvious differences of detail. Thus the epidermal cells of the hybrid are a little larger, although not nearly as large as those of *P. aureum* (Fig. 9), but the guard-cells of the stomata closely resemble the guard-cells of the last-mentioned plant, save in their smaller size.

The roots of the three Ferns show a complete series of gradations, those of the hybrid standing, on the average. exactly halfway between the thin ones of *P. elegantissimum* and the much thicker ones of *P. aureum*. But the degree of variation in the detailed structure of the individual roots of even one specimen renders these organs less suitable than the rest for our present purpose, and they will not be further considered here.

Turning now to the sporangia, I have already alluded to the dimorphic character of the leaves in both *P. elegantissimum* and *P. Schneideri*. In the former Fern the sori are found far more abundantly on the plain than on the dissected fronds, and, when plain pinnae occur on a dissected frond, the sori are far more abundant on the uncut portions (Pl. XXIV, Fig. 15*a*); still the sporangia of both leaf-forms produce germinable spores, and indeed it was from spores of these dissected leaves that the parent prothallia were grown. In the case of *P. Schneideri*, however, it is rare to find sporangia upon the dissected leaf at all, and when they do occur they are quite barren. Mr. Schneider tells me he has never succeeded in raising any prothallia from them, and his statement is borne out by the fact that in all the specimens I was able to examine I found the spores degenerated. The abortion frequently does not become apparent until the spore-mother-cell, or even the tetrad, stage is reached; but in any case the mature sporangium only contains a granular mass resembling a heap of angular composite starch-grains.

In marked contrast to the scarcity of sporangia on the normal fronds of *P. Schneideri* stands their extreme abundance on the reverted leaves; even any odd pinna of a normal frond which exhibits a plain instead of a cut outline is commonly crowded with sori (Pl. XXIV, Fig. 18). And yet it is quite certain that the majority at any rate of these sporangia are sterile. Some appear, notwithstanding, to possess good spores, but they have not yet been successfully germinated. Mere appearance, however, forms but an unsafe guide in estimating germinating power, as is proved by the case of *Adiantum Farleyense*. This Fern is, on good grounds, believed to have arisen as a sport from *A. tenerum* in Barbados, and although it sometimes bears sporangia containing spores which are to all appearance good, they are said, by the best authorities competent to judge, to be incapable of germination. It is known that vigorous sports are sometimes highly sterile, and this circumstance, coupled with the frequent luxuriance in vegetative development of sterile leaves, has given rise to a doctrine of correlation which is doubtless a neat way of expressing the facts, but which in reality leaves us exactly where we were before. It seems, however, not altogether improbable that an extended experimental study of dimorphic Fern-leaves might well prove to be one of the avenues leading to a clearer conception of the essential differences between what we at present term vegetative and reproductive activity.

Regarding the plant now under discussion from another aspect, there are some interesting points of contact between it and other hybrids, as for example the famous *Cytisus Adami*.

The latter, as is known, is a hybrid between the arborescent *C. Laburnum* and the shrubby *C. purpureus*, although it is by no means agreed as to whether it arose as a seminal or a graft hybrid. The authority for the latter origin rests chiefly on the statements made by Adam, and on the failure of all attempts to cross the two parent forms.

The *C. Adami* produces on the same plant flowers and shoots which may be roughly referred to three categories[1]:—

1. The hybrid flowers, intermediate between the two parents, and often highly variable.
2. Flowers closely resembling the *Laburnum*, and these are commonly borne on specially vigorous shoots.
3. Shoots with flowers and leaves of the *purpureus* type.

Now the regular *Adami* flowers are sterile. The pollen is indifferent, and the ovules, according to Caspary, are monstrous and incapable of fertilization. But the two reverting forms can both set good seed from which new plants can be raised. And although the seedlings thus obtained closely resemble the parent form to which the seed-bearing shoot had reverted, it is clear that they have not purged themselves of their mixed origin, since Herbert[2], while hinting at a certain amount of variability amongst the *purpureus* lot, expressly mentions the fact that a purple colour was sometimes present on the flower-stalks of seedling-plants which otherwise would be referable to *C. Laburnum*. The fact that the reversion to the parent form in *C. Adami* is both more perfect and also seems to be accompanied by a corresponding increase in fertility, when compared with the state of things present in *P. Schneideri*, does not in any way affect the legitimacy of instituting a comparison between these two plants; but rather adds to its interest.

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[1] See description (with coloured plates) by Édouard Morren in La Belgique Horticole, 1871.

[2] Herbert, Journ. Hort. Soc., vol. ii.

Although the reversion on the part of the Polypody is commonly in the direction of the *vulgare* type, indications are not wanting, as has already been stated, that the *aureum* form may also reassert itself; and in the *Cytisus* it is usual for one (commonly the *Laburnum*) parent to manifest itself before the other. The extreme sterility of the hybrid type in both cases is of special interest when contrasted with the known fertility of the reverted portions of *Cytisus* and their possible fertility in the Fern. Even should the spores of the latter prove not to be germinable, they are, as microscopic examination shows, far less hopelessly barren than are those on the hybrid plants, and they are immeasurably more numerous.

Of course it may be argued, as has already been hinted, that the sterility of the hybrid leaf is not necessarily a consequence of its hybrid origin, but is merely correlated with the foliar expansion, just as the leafy sterile part of an *Osmunda* frond is said to be when compared with its contracted fertile part. And it might be urged that, on my own showing, the dissected fronds of *P. elegantissimum* are not so sporangiferous as are those which have assimilated themselves to the *vulgare* type. But the first argument, even if the premises be admitted, is not to the point; and with regard to the second, it may be said that we are thereby brought no nearer to accounting for the absolutely *sterile* nature of the hybrid spores, whether the sori are produced in abundance or not, and this peculiarity must, I think, depend on some intimate change in the configuration or composition of the protoplasm, and I believe we may here fairly ascribe it to the effect of the crossing. Although it may be premature to speculate on the nature of the processes concerned in the production of hybrids, I am convinced that a careful study of these organisms will do much to throw light on the obscurities of heredity, and perhaps even on the essentials of ordinary fertilization. It seems certain that the quality which we term heredity, by virtue of which organisms are what they are, and what their forbears have been before them, must either be associated with definite discrete particles, ids, which by a series of kaleidoscopic rearrangements are able to form new combinations after the manner imagined by Weismann: or else that heredity must depend on the configuration, physical and molecular, of a *substance taken as a whole*, and its interaction with the other constituents of the cell. In the former case, with each generation a new arrangement of the pre-existent and persistent particles, each associated with hereditary qualities, is assumed to occur; in the latter case a new substance must be formed, which, by virtue of its constitution *as a whole*, governs and determines the mode of development of the organism. The id-theory seems irreconcilable with what we now know of the details of fertilization in plants, and at least in some animals. In these, what direct evidence we have, tells against the preliminary elimination of unlike hereditary units from the sexual cells during their course of development, and clearly indicates rather an equivalent distribution of substance. But if this is really true, even in a few cases, the objective basis of the id-theory is clearly destroyed; for the elimination of half the parental ids from each gamete previous to their union, forms an essential part of the theoretical structure built up and maintained by Weismann and his followers. The case of hybrids has been used by the exponents of the id-theory to strengthen their case, and preponderating multiplication of paternal or maternal ids—the result of an imaginary struggle between purely hypothetical combatants—is supposed to finally account for the resemblance of this or that cell to one or other parent; but the phenomena seem to be more readily susceptible of explanation if we assume that the hereditary substance acts *as a whole*, and by virtue of its own particular composition. The hereditary substance derived from two parents might equally well form a relatively stable or an unstable combination whether this depended on the nature of the molecular structure of the substance in question or on still more complex arrangement of molecular aggregates. In any case, operating, as it must, on and through the rest of the cell-contents, the results of its activity will be profoundly modified by the nature of the latter, and also, though less immediately perhaps, by such external agencies as in turn may affect them. And thus I think we may in a measure see how a plant can become materially altered in its form so as to suit different environments, without having to postulate the existence of a reserve store of emergency ids in order to explain the often extraordinary manifestation of adaptive variations which may arise within the limits of a single species or even of a single individual. In this connexion I might cite, for example, the variation of the leaf-form[1] met with in amphibious plants. We know of many cases in which a plant will develop in a particular way as the result of stimuli which we can hardly regard as other than physical or chemical, certainly not as the result of the direct action of hereditary ids. Thus pollination is a necessary antecedent to ovule-formation in many flowers. But if it be urged that the ids are there, and only need the stimulus to excite them to activity, this explanation can scarcely be seriously urged to meet the case of galls, each form of which is peculiar to and characteristic of a special parasite reacting on a special plant. Again, in the St. Valéry apple, different forms of fruit are produced on the same tree as the result of pollination from different sources of apples. Here there can be no question as to the travelling out of the ids from the new generation into the sporophyte of the old, whereby a particular new form of fruit is stimulated to grow. Nor can we assume that the tree had stores of ids requisite to produce this or that form of fruit. If we say that the pollination stimulated the formation of new arrangements of ids, we are going far beyond analogies based on knowledge, which would rather suggest chemical interaction, and are simply abandoning ourselves to flights of pure fancy.

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[1] Weismann has discussed the question of polymorphism (*The Germ Plasm*, Contemporary Science Series, 1893), with the result of adding considerably to the complexity of his theory.

And to return to the Fern, I confess I find it almost impossible to imagine a machinery complex enough to account for the reversions it exhibits on the assumption of separate ids, whilst it does not, to me at least, seem so unintelligible on the alternative view. Instances might easily be multiplied. Thus Hildebrand[1] found, in a cross between *Oxalis latifolia* and *O. tetraphylla*, that the characteristically distinct hairs of each species might both arise from a single epidermal cell of the offspring. Are we in this case to imagine that the ids and determinants of the corresponding cells in both parents were all actively present in the nucleus of this epidermal cell? And if we answer in the affirmative, how shall we explain the further case that amongst the offspring obtained by crossing *O. lasiandra* with *O. Andreuxii*, some of the hybrids possessed flowers readily distinguishable from those of either parent, not only on account of their larger size, but also in their totally different colour? What, we may ask, has become of the action of the individual ids here? The frequently observed sterility of hybrids, as well as the failure of numerous likely crosses to set seed, may be due, quite as well as to an unsuitable consorting of ids, to the imperfect combination or union of the parental substances, a defect the results of which (whatever its true nature may be) are apparent in all degrees and gradations varying from positive and absolute sterility on the one hand and only stopping short of actual fertility on the other.

These are only tentative suggestions, for in the present condition of our knowledge we can only grope about after a clue to the solution of the problems before us—a clue which recent cytological work seems to have shown us to be less accessible than we had previously hoped. But I do not believe they are really unsoluble; they demand, however, experimental treatment on the part of botanists such as they have already begun to receive at the hands of comparative physiologists, and it behoves cytologists not to neglect such means of assistance as a careful study of the structure and origin of hybrids, in the garden as well as in the laboratory, is able to provide.

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[1] F. Hildebrand, Ueber einige Pflanzenbastardierungen; Jen. Zeitschr., Bd. 23, 1889.

EXPLANATION OF FIGURES IN PLATES XXIII AND XXIV.

Illustrating Professor Farmer's paper on the Structure of a Hybrid Fern.

[Figs. 1–13, camera lucida drawings, Zeiss A × 2 oc. (the figures 1–9 reduced to ½). Figs. 14–19, photographs, ¾ natural size.]

Figs. 1, 2, 3. Sections of the petiole of *Polypodium aureum*.

Fig. 1. Transverse section through the base of the petiole, showing peripheral band of sclerenchyma, *scl.*, and the steles, two large and five small ones. *e.* endodermis; *P.* pericycle; *ph.* phloem; *xy.* xylem.

Fig. 2. Section a little below the middle of the petiole. Shows anastomosis between the smaller steles.

Fig. 3. Section a little below the region of the lamina of the leaf.

Fig. 4. Section through the base of the petiole of *Polypodium vulgare*, var. *elegantissimum*.

Fig. 5. Section taken through the same petiole at ⅓ the distance between the base and the insertion of the lowest pinna.

Figs. 6, 7, 8. Sections through the petiole of *Polypodium Schneideri*, taken from regions corresponding with those of *P. aureum* in Figs. 1–3.

Fig. 9. Epidermis from the lower surface of the leaf of *Polypodium aureum*.

Fig. 10. Epidermis from the lower surface of the leaf of *P. vulgare*, var. *elegantissimum*.

Fig. 11. Epidermis from the lower surface of the leaf of *P. Schneideri*.

Fig. 12. Sporangium (showing barren contents) from dissected frond of *P. Schneideri*.

Fig. 13. Sporangium from 'reverted' frond of the same fern.

Fig. 14. Leaf of *P. aureum* (under side).

Figs. 15, 15 *a*. Two leaves of *P. vulgare*, var. *elegantissimum*. Both show 'reverted' pinnæ.

Fig. 16. Normal frond of *P. Schneideri* (under surface).

Fig. 17. Partially 'reverted' leaf of same (upper surface).

Fig. 18. Under surface of a similar leaf. Sporangia abundant on 'reverted' parts.

Fig. 19. Completely 'reverted' leaf of the same. Sporangia very abundant.

Fig. 1.

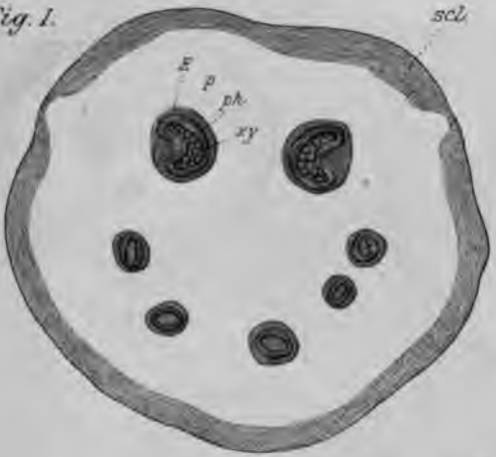
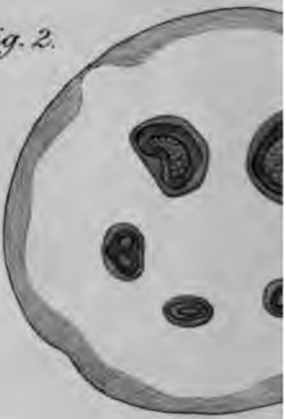


Fig. 2.



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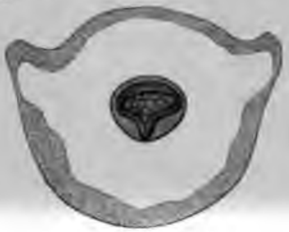

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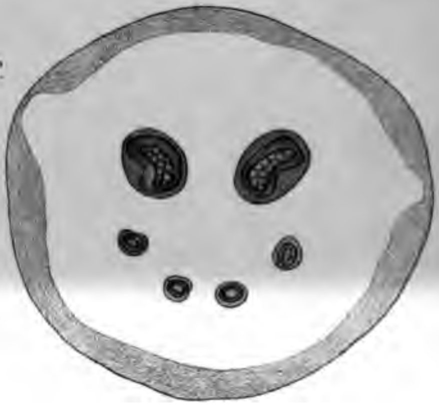

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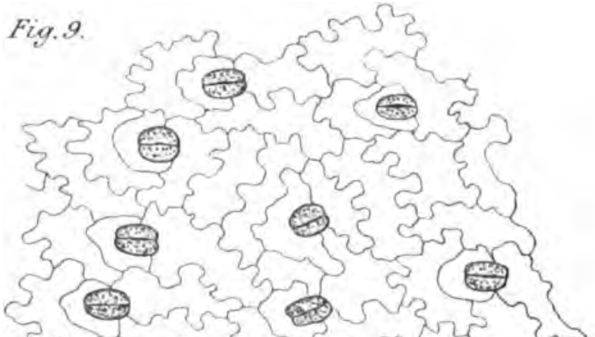
Fig. 9.



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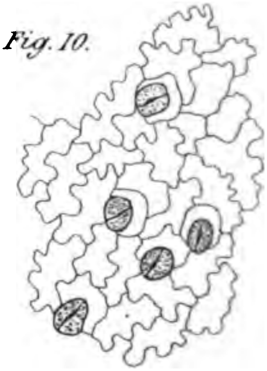

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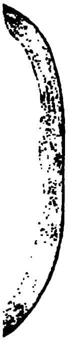
FARMER. — HYBRID FERNS.



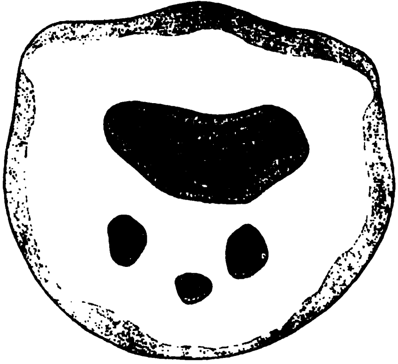
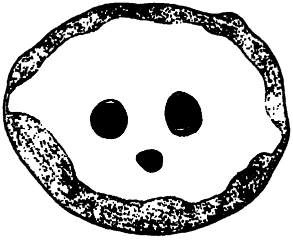


Fig. 3.



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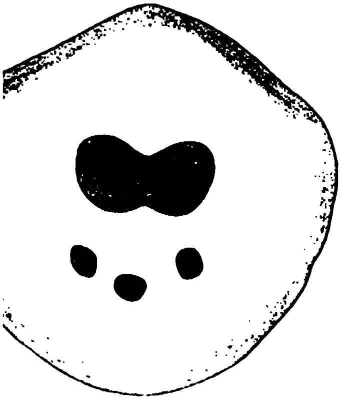


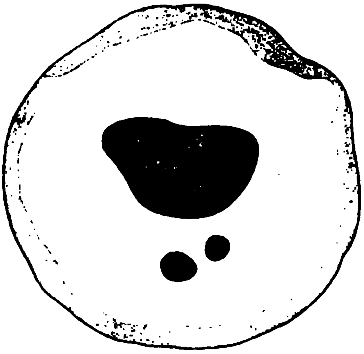
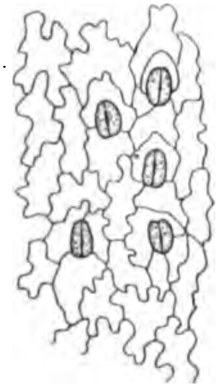


Fig. 8.



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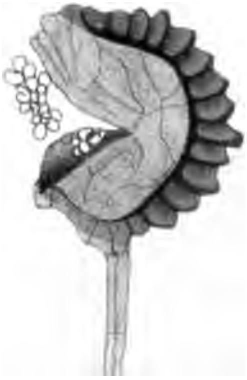

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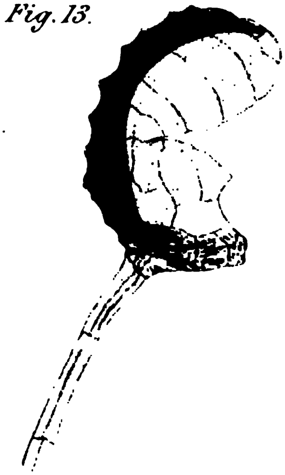
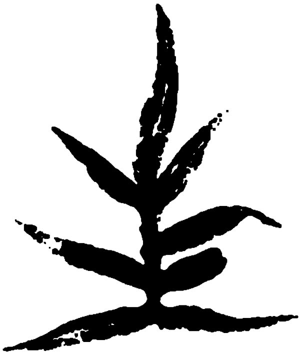


Fig. 13.

University Press, Oxford.



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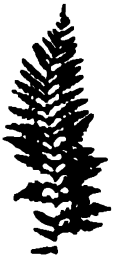

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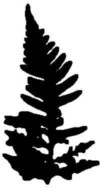

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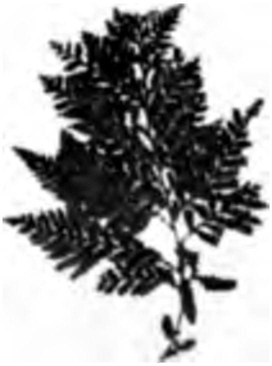

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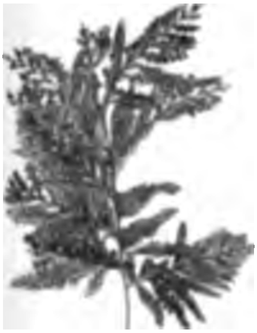

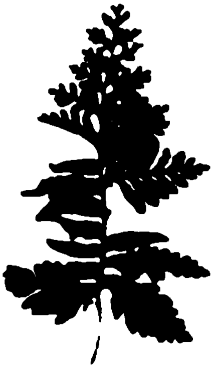

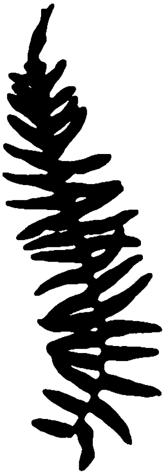

Fig. [illegible]

FARMER. — HYBRID FERNS.

The Antherozoids of Dictyota and Taonia.

BY

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With Plate XXV.

THE antheridia of *Dictyota dichotoma* (Huds.) Lamour. were first described by Thuret[1]. The paper is accompanied by excellent figures of the antheridia in various stages of development. With regard to the antherozoids he states that when they are liberated 'they spread out in the water in the form of hyaline globules like the spermatia which escape from the antheridia in the Florideae and they seem, like them, non-motile.' Crouan[2] professed to have seen motile gametes (called by him sporidia) in *Halyseris polypodioides*, Ag., but his description evidently applies to the oospheres and not to antherozoids. Thuret also expressed disbelief in the truth of his statement. Reinke[3] has given detailed descriptions of the genera *Dictyota*, *Padina*, *Taonia* and *Zonaria*. He confirms the statement of Thuret with

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[1] Ann. d. Sci. Nat., 4e sér. Bot., t. III, 1855.

[2] Bull. de la Soc. Bot. de France (1855), t. II, p. 444.

[3] Nova Acta Leop.-Carol., Bd. XL, 1878.

[Annals of Botany, Vol. XI. No. XLIV. December, 1897.]

regard to the form and non-motility of the spermatia, and his figures are almost identical with those of the earlier botanist. In 1889, Johnson[1], on the strength of observations made on *Halyseris*, expressed his belief that the so-called spermatia were motile antherozoids. His reasons are thus stated: 'On one occasion, between ten and eleven in the evening, I was examining the antheridia microscopically when I saw one from which the male corpuscles were escaping and exhibiting movements of such a nature as to give me the impression that they were ciliated. Subsequent examination, again and again repeated in different ways (and with $\frac{1}{16}$ mm. immersion objective), of preserved material has strengthened my conviction that the male corpuscles of *Dictyopteris* are not pollinoids like those of the Florideae, but antherozoids, essentially like those of Cutleriaceae and Fucaceae. Fig. 10, Pl. V, representing a developing antherozoid of *Fucus vesiculosus* in Guignard's paper[2], is very like stages I have seen in the developing male corpuscles of *Dictyopteris*. Still I must ask to be permitted to reserve a final expression of opinion on the presence of cilia until I have made a detailed examination of fresh material.' This observation has not been confirmed either by Johnson himself or by any other algologist, so that in all the text-books, the possession of non-motile spermatia is still regarded as one of the distinguishing characters of the Dictyotaceae.

My attention became directed to the Dictyotaceae during the progress of a joint investigation[3] into the details of fertilization in the Fucaceae. It was found desirable to test the correctness of Thuret's view, that parthenogenesis obtained in the group. If this view were proved incorrect, then the further question arose whether fertilization took place as in the Fucaceae, outside the parent plants, or before the liberation of the oospheres, as suggested by Reinke[4].

On Sept. 8, 1896, early in the morning, I placed in several watch-glasses and capsules of sea-water pieces of antheridial plants of *Dictyota*. On examining the vessels after a short interval, to my astonishment, they were all full of actively moving antherozoids. I watched them as long as they remained active, but found that they came to rest much sooner than antherozoids of *Fucus* would have done.

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[1] Linn. Soc. Jour. XXVII, 1891.

[2] Rev. gén. de Bot., I, 1889.

[3] Proc. Roy. Soc., Vol. 60, p. 188.

[4] Loc. cit.

Unfortunately I was unable to resume my observations till Sept. 16. By that time the high spring tides were over and the plants had to be dredged up. When examined, the plants were found to have liberated all their mature sexual elements, but there was a large number of young stages. More plants were obtained on the 17th, 18th and the 21st. It could be easily seen that the antheridia and oogonia were getting more and more mature, but I had to leave the seaside without getting plants that were ready for the liberation of either male or female elements. Under the circumstances it was thought better to wait for another season so as to obtain full confirmation before publishing the observation.

During the present summer several attempts were made to secure plants with mature antheridia, but without success until Aug. 21. Male plants collected the previous afternoon, and kept in the dark all night, were in the morning placed in sea-water. There was an immediate liberation of antherozoids which were quite as active as those of *Fucus*. In general appearance they seemed at first sight strikingly similar to the latter, but a closer examination showed that, both in the form of the head and in mode of locomotion, there were minute differences which served to distinguish them from the latter; and after fixation and staining, still other and more remarkable differences became perceptible.

Fragments of tetrasporous and oogonial plants were also subjected to the same treatment, but at no time could swarming organisms be seen in the water in which they had been placed. Here it might also be added that, in the later observations made on antheridial plants, these actively moving bodies were never obtained except from such as had mature antheridia, and not even then, unless treated in some such manner as above described. Any doubts, however, which might have arisen, as to the swarming organisms being the antherozoids of *Dictyota*, were set at rest by the direct observation of the contents of the antheridia starting into activity and swimming away. This could easily be seen by focussing the microscope on the surface of a sorus.

The following is a summary of the principal points observed in connexion with the antherozoids.

If mature antheridial sori are examined, the surface is seen to be white and glistening. Each antheridium is divided into a large number of small cells, the contents of each cell forming a single antherozoid. These at first seem to present no differentiation of structure, but a closer examination shows the presence of a small round globule in each of the cells, situate either at the centre or near the periphery. After fixing and staining the antheridia, this spherical body is not always evident. The greater part of the circumference of the antherozoid stains deeply, showing the presence of denser protoplasm irregularly disposed in roundish or elongated masses which occasionally cross the surface of the cell. The cause of this appearance will be discussed later on. With regard to the spherical bodies above mentioned, it may be further added that in some cases a very faint tinge of yellow is observable in them. This suggests the possibility of their representing the 'chromatic spot' characteristic of the antherozoids of the Fucaceae. Though smaller and very much less obvious than the latter, they are quite easily recognizable in the antheridia of *Dictyota*, *Taonia*, and *Halyseris*. In motile antherozoids they very often seem to project beyond the periphery of the head, as also do the eye-spots in the antherozoids of *Fucus*. The study of fixed material, however, makes it rather doubtful that these granules represent 'eye-spots.' In the first place they are not invariably present, and even where they occur, their position has no definite relation to the points of insertion of the cilia. My opportunities for collecting during the past summer have not been many. It has also been difficult to secure all the conditions necessary for the exhibition of motility in the antherozoids, the weather in particular having been very unfavourable on account of its dulness and coldness. Even when the antherozoids were active, the shortness of their period of motility rendered it difficult to make a study of their structure.

When the antheridia are mature, the walls dissolve and liberate the antherozoids. If the light is not bright enough, or the temperature is too low, or the plant is not sufficiently vigorous, they passively float away and get distributed through the liquid without showing any signs of motility. This has been described by Thuret, Reinke, and other observers. If, however, the plants be kept over-night in damp air in a darkened chamber, and then placed in sea-water, there is no difficulty in getting the male corpuscules to swarm, provided it is sufficiently bright and not too cold. This is interesting in view of the attempts of Savaugeau[1] to repeat the observations of Berthold on the conjugation of planogametes in *Ectocarpus siliculosus*. He found that this phenomenon was only observable early in the morning, and not even then if the sky were dull and cloudy.

While actively moving, the body of the antherozoid is seen to be somewhat similar to that of *Himanthalia* or of *Cystoseira*. It has not the sharply pointed end so characteristic of *Fucus*, and particularly of antherozoids of *Pelvetia*; after fixation it becomes perfectly spherical, but during active movement it is seen to be somewhat elongated, with one end frequently narrower but quite blunt. The movement itself, though at first sight similar to that of antherozoids in Fucaceae, has peculiarities of its own, which, after a careful examination of stained material, we can easily correlate with the character of the cilia.

In most of the experiments, the motility of the antherozoids continued for only a short time, about ten minutes being the average duration. In other cases, however, some have continued in motion for as long as forty minutes.

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[1] Journ. de Bot., X, 1896.

On one occasion I was examining antheridial plants about 3 p.m. The liberation of antherozoids was so great that the slide was covered with them, but there was no sign of activity to be seen anywhere among them. The microscope was taken to a window on which the sun was shining and some of the rays were caused to pass through the slide. In a very short time the antherozoids were swarming like bees on a hot summer's day. On examining other parts of the slide it was seen that motility was strictly confined to the portion directly exposed to sunlight. There the swarming continued for a time, but stopped entirely at the end of ten minutes from its commencement. This observation shows that these bodies are highly sensitive to light and that they can only exhibit active movement under the influence of this stimulus.

Want of opportunity prevented my making direct observation on their phototactic characters, but the following may be of some interest in this connexion. One evening I was examining a half-empty sorus. The light used was that of a small oil-lamp. In the cavity of the sorus a few antherozoids were floating and showing a scarcely perceptible motion. I observed that all were slowly sinking in the cavity, at the same time changing their places relatively to each other. The light was turned off for a short time and then it was found that they had re-ascended. This experiment was several times repeated with the same result, showing that in this particular instance, where the light was not very strong and the antherozoids not vigorous, the phototaxis was positive.

Several lots of antherozoids were fixed, the best results having been obtained with dilute Flemming's solution. All were stained with Hoffmann's blue, either in dilute glycerine or after drying on the slide according to the bacterial method. In the latter preparation the cilia are particularly well shown. Contrary to what obtains in all other Phaeophyceae hitherto described, instead of there being two cilia, each antherozoid has a single very long flagellum. When fully extended, as it is in the dried preparation, the flagellum often extends to six times the diameter of the head. It has to be borne in mind that in the spermatozoids of *Himanthalia* and *Cystoseira*, which as already stated are very similar to those of *Dictyota* in the shape of the head, there is great disparity in length between the two cilia, one being very short while the other is correspondingly long. The possibility suggested itself that this might be the case here, one being so much reduced as to be almost invisible. In spite, however, of the greatest care taken in observing, no trace of a second cilium was seen. The probability that there is only one flagellum to each antherozoid is strengthened by the following facts:—

1. The flagellum in the great majority of cases proceeds in a radial direction from the head (see Fig. 2) instead of being directed tangentially to it as in the Fucaceae. Antherozoids of *Himanthalia* were fixed and dried for comparison. In spite of the rough treatment the biciliate character and the tangential attachment were clearly shown.
2. In the glycerine-preparations the flagellum is strikingly spiral (Fig. 3); this never occurs in the Fucaceae.
3. As already stated, the character of the movement was suggestive of the presence of a single flagellum.

When the glycerine-preparations are examined it is seen that instead of consisting of hyaline protoplasm, as described by Thuret[1], the head of each antherozoid shows clear and unmistakable differentiation of structure. The greater part of it appears clear and transparent, while one side is occupied by a thin layer of denser protoplasm staining deeply with Hoffmann's blue. When viewed in profile this seems crescentic in form, with the inner border often wavy but always sharply defined. The comparison of a number of antherozoids lying in different positions shows that this denser protoplasm forms a lining of approximately circular form but with an irregular, wavy, often indented border. It may be compared to a watch-glass with an irregular, indented rim (Fig. 3). The small spherical body already mentioned is most often close to the dense protoplasm; sometimes it lies in the centre of the concavity, either resting upon the surface of the layer or imbedded in its substance, and even occasionally projecting beyond the general surface of the head. In some cases, however, it lies away from the dense layer altogether.

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[1] Loc. cit.

The flagellum arises from about the centre of the disk of denser protoplasm. In a few exceptional cases it seems to be attached to the edge; but this is probably due to the first coil of the spiral flagellum lying close to the head so as to be partly covered by it (Fig. 3).

Some of the antherozoids were fixed when the motion was becoming sluggish. When these were stained it was found that the flagellum in each case was more or less closely coiled round the head. Some typical examples are shown in Fig. 4. This naturally suggests that the reason for the difficulty of seeing the flagellum in antherozoids which are not motile is, that it is closely coiled round the head. The same fact may account for the apparently greater amount of deeply staining protoplasm in the contents of the antheridial cells than in the head of the swarming antherozoid.

The same phenomena were observed in male plants of *Taonia atomaria*, J. Ag., collected at Llandudno, but in this case no permanent preparations were made.

I beg to express my thanks to the Council of the Royal College of Science for allowing me the use of a free table in the Botanical Laboratory, and to Professor Farmer for valuable assistance and advice. I am also indebted to Professor Phillips and the Council of the University College of North Wales for the privilege of using the Botanical Laboratory while collecting material in the Menai Straits.

ROYAL COLLEGE OF SCIENCE, LONDON,
Sept. 30, 1897.

EXPLANATION OF PLATE XXV.

Illustrating Mr. Williams' paper on the Antherozoids of *Dictyota* and *Taonia*.

All the figures relate to *Dictyota dichotoma*, Lam.

Fig. 1. Part of a sorus of antheridia in vertical section. Fixed in Flemming's mixture and stained with Hoffmann's blue.

Fig. 2. Antherozoids fixed as above, dried on the slide, then stained with Hoffmann's blue and mounted in balsam.

Fig. 3. Antherozoids fixed while very active with Flemming's mixture, stained in Hoffmann's blue and mounted in dilute glycerine. (Camera lucida drawings).

Fig. 4. Antherozoids fixed when very sluggish, dried and stained in the same way as Fig. 2.

Fig. 1.

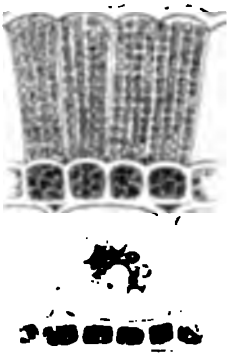


Fig. 4.

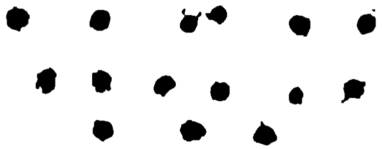


Fig. [illegible]

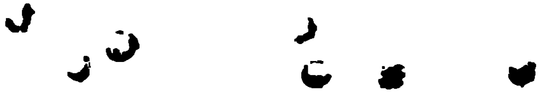
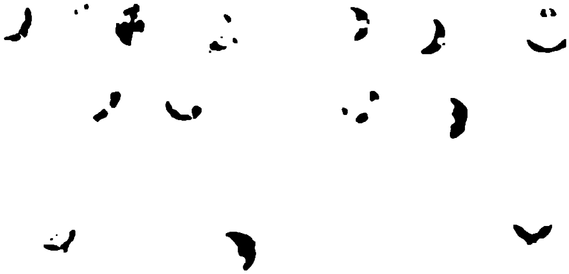


Fig. [illegible]



[illegible]

WILLIAMS — DICTYOTA.

The supposed Alcoholic Enzyme in Yeast.

BY

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EARLY in the present year a paper was published by Dr. E. Buchner (1), in which he stated that he had succeeded in extracting from Yeast a liquid possessing the power of setting up the alcoholic fermentation in solution of cane-sugar. His method was the following:—One kilogram of Yeast was dried by pressure until it formed a friable powder. When so dried it was mixed with an equal weight of fine quartz sand and with 250 grams of a fine infusorial earth (Kieselguhr), and the whole carefully ground in a mortar. Water was added to the fine powder, now become pasty owing to the breaking up of the Yeast-cells, 100 cc. being used to the kilogram of Yeast. The mixture was then wrapped in a cloth and gradually submitted to strong pressure in a hydraulic press, the pressure being worked up to 400–500 atmospheres to the square inch. The resulting liquid measured about 300 cc. The cake, when removed from the press, was ground up again, and the powder extracted with a further 100 cc. of water. This was again subjected to the action of the press, and a further 150 cc. of liquid was obtained. Each kilogram of Yeast thus furnished about 450 cc. of expressed liquid, of

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which only 150 cc. represented added water, and the remainder was extracted from the living Yeast-cells.

This turbid liquid was then mixed with four grams of the same Kieselguhr and well shaken. On filtering it through paper a clear yellow liquid was obtained, having an aroma like that of active Yeast.

This liquid was the material in which Buchner claimed that the long-sought alcohol-producing enzyme was present. It had a specific gravity of 1·0416 at 17° C.

In its properties it closely resembled solutions of the other enzymes already known to physiologists. It lost its power of inducing fermentation on being heated, but parted with it at a much lower temperature than most enzymes, viz. at 45–50° C., its proteids being coagulated at the same temperature. The coagulum was very bulky, the extract being extremely rich in proteid matter. The coagulation was preceded by the evolution of carbon dioxide.

When this Yeast-extract was mixed with a 37% solution of cane-sugar, a regular evolution of carbon dioxide commenced, and proceeded with an energy which varied according to the temperature to which the liquid was exposed; it was active even so low as 0° C., but became more obvious at the temperature of the laboratory, and still more energetic at 40° C. The extract was found to work upon the same sugars as the Yeast itself, and to refuse to attack those on which the cells had no action.

The activity noticed was not interfered with by chloroform or other antiseptics, nor was it abolished by filtering the extract under pressure through a porcelain filter. There was thus a strong presumption at least that no living Yeast-cells were present in the extract, and that whatever happened was due to something extracted from the cells by the enormous pressure employed in the process.

In a subsequent paper (2) Buchner admits that this active principle is not contained in all Yeasts, the so-called 'German Yeast' being free from it. He further states that the active liquid can only be preserved for a single day at the ordinary temperature of the laboratory or for two days at 0° C. The inactive liquid which gave a bulky coagulum at 45°–50° C. when gradually heated, while in possession of its powers, seems to lose the power of forming the coagulum as its fermentative property disappears. Buchner suggests that the supposed enzyme is proteid in character, and is digested rapidly in the liquid, with the other proteids it contains, under the influence of peptic enzymes extracted from the cells.

Buchner says further that if the Yeast be very carefully dried, it can be heated for six hours to a temperature of 100° C. without destroying the power of the Yeast to induce fermentation. This temperature is sufficient to kill the cells, but, according to the author, is not high enough to destroy the enzyme they contain. Hence a fermentation can be obtained similar to that set up by his Yeast-extract.

The appearance of these researches excited a good deal of interest, especially among physiologists and chemists, the more so as the Yeast-plant has always been the chief support of the vitalistic theory of fermentation put forward by Pasteur. It is only natural that researches have been undertaken by many workers with the view of confirming or disproving Buchner's statements. I have for several months been at work upon the subject, and have embodied my experiences in the present paper.

On reading Buchner's account of his experiments it appears that he lays great stress on the evolution of carbon dioxide from the mixtures of his extract with the various solutions of sugar which he used. To him such an evolution of gas was evidence of fermentation, and his deduction was that an enzyme was at work in his liquids. There are, however, two other points to which attention may fairly be called in conducting the experiments. The sugar-solution should gradually become of a less specific gravity as the fermentation proceeds; conditions being constant, this diminution of specific gravity should be regular as well as progressive. Also there should be a gradual and continuous formation of alcohol in the liquid as the fermentation proceeds. If a true fermentation is set up, not only should these three phenomena be capable of separate demonstration, but there should be a definite quantitative relation between them.

I have carried out several series of experiments with different Yeasts, using in the first place the ordinary high fermentation Yeast used by our local brewers at Cambridge. I have made one series on a sample of low fermentation Yeast kindly supplied me by my friend Mr. Armstrong, of the Tottenham Lager Brewery, London.

I followed Buchner's method of preparation of the extract as closely as I could. In the process of grinding up the Yeast-cells with Kieselguhr and fine sand, I examined each instalment in the mortar with a $\frac{1}{8}$-inch objective, and kept on the grinding till about 75 to 80°/ₒ of the Yeast-cells were ruptured. My extracts differed from his in quantity, never measuring so much as his figures led me to expect. In physical peculiarities there was a very close correspondence between us, my preparations coagulating at the stated temperature, and possessing the proper colour and smell.

On mixing the extract and the sterilized sugar-solution, the latter being sometimes solution of cane or grape sugar, and sometimes a wort obtained from the brewery, I have always failed to observe the copious evolution of gas which Buchner speaks of. I carried out the experiments in freshly sterilized Ehrlenmeyer flasks fitted with a mercury-manometer. Instead of a rise of the mercury in the distal limb, soon after the experiment was started there was almost uniformly a rise in the proximal limb, indicating an absorption and not an evolution of gas. In no case did I ever get a measurable rise of the mercury in the distal limb of the manometer unless the liquid contained some Yeast-cells that had been imported into the flask. This happened occasionally, even filtration through porcelain and the addition of chloroform sometimes failing to prevent it.

Not being able to get the copious evolution of CO_2, I turned my attention to the specific gravity of the fermenting solution. It seemed possible that a small quantity of sugar might be undergoing decomposition, but that the mercury-manometer was not sufficiently delicate to give evidence of it.

In the bulk of my experiments I relied on the plan of taking the weight of a specific-gravity-bottle full of the fermenting liquor at the same temperature at approximately regular intervals. The same course of action could generally be observed, and I will therefore only quote two typical observations.

The first of these extended over a period of about ten days, the fermenting liquid being a solution of cane-sugar ($40^{\circ}/_{0}$) mixed with an equal volume of the Yeast-extract. This was filtered twice through porcelain before being experimented with, and the flask was allowed to stand in an incubator at 33° C. The weighings were taken in a sp.-gr.-bottle of 25 cc. capacity. The successive weights are given in the following table:—

Date.	Time of day.	Hours of fermentation.	Weight in gms.	Loss.	Loss per hour.
May 5	12 noon	—	40·7445	—	—
„ 6	11 a.m.	23	40·7385	·006	·00026
„ 7	5 p.m.	30	40·732	·0065	·00021
„ 8	5 p.m.	24	40·726	·006	·00025
„ 10	11 a.m.	42	40·723	·003	·000075
„ 11	11 a.m.	24	40·722	·001	·000041
„ 12	5 p.m.	30	40·715	·007	·00023
„ 15	5 p.m.	72	40·702	·013	·00018

During the first three days the course of action resembled that of a weak enzyme, and appeared to confirm Buchner's views. After six days the activity had fallen to one-sixth the original amount, which again would not be surprising. During the next few days, however, it went up again to the original amount, which was quite contrary to one's experience of the action of enzymes.

In the second experiment I wish to quote, the extract was mixed with its own volume of $40^{\circ}/_{0}$ solution of glucose and digested in an incubator as before. The 25 cc. weighed 40·0915 gms., and after thirty hours' digestion it fell to 40·0905, showing a loss of ·0003 gm. per hour. After this time it gained in weight, and the mercury in the proximal limb of the manometer rose slightly, pointing to an absorption of some constituent of the air in the flask.

Dr. Blackman was kind enough to measure for me, by means of his very delicate apparatus (described by him in the Phil. Trans. (3)), the amount of CO_2 that was being evolved by the fermenting liquid per hour in both of these experiments. The quantity varied very slightly from time to time, did not show a diminution *pari passu* with the diminution of the weight of the sugar-solution, and did not amount to more than ·01 cc. per hour, which is not more than one-tenth of the quantity which would have been produced had the loss of weight of the sugar-solution been caused by the splitting of a corresponding amount of sugar into CO_2 and alcohol.

At the end of the experiment I examined the digestions for alcohol in the usual way, by neutralizing, distilling off two-thirds of the liquid, adding distilled water to make up the original bulk, and taking the specific gravity. After the ten days of the first experiment quoted, the liquid contained ·2°/₀ of alcohol.

I may quote further a series of experiments I made to ascertain if alcohol was produced during the fermentations, and whether, if so, the quantity increased in any proportion to the duration of the digestion.

I mixed 50 cc. of a freshly prepared Yeast-extract with 100 cc. of a 40°/₀ solution of glucose, and divided it into five equal parts, A, B, C, D, and E, which were set in an incubator at 29° C. in flasks fitted with gauges or manometers. Prior to starting the digestion A was boiled to serve as a control. It gave the usual bulky coagulum at 45° to 50° C. The experiments were started on May 28, and the weight of a specific-gravity-bottle filled with the liquid was 27·350 gms. One flask, C, was examined at intervals of twenty-four hours, and the weights obtained were the following:—

May 31, 27·338 gms.
June 1, 27·340 „
„ 2, 27·338 „

The contents of this flask were then examined for alcohol, and found to contain ·2°/₀.

B was allowed to digest for three days longer. It then contained the same proportion of alcohol. When boiled it gave hardly any coagulum, the proteids being apparently digested, as Buchner says they were in his experiments.

E was examined after four more days. It contained almost exactly the same percentage of alcohol.

Two days later, the control flask A was similarly examined, and found to contain the same amount of spirit.

There had thus been during the whole time no additional amount of alcohol formed.

From a consideration of the whole series of my experiments I can see no satisfactory evidence in favour of the existence of Buchner's enzyme, at any rate in the Yeasts in use in English brewing. The diminution in specific gravity in the digesting fluid in the quoted series of experiments appeared to me at first to point in that direction but had the enzyme been present I think I should have found this diminution more regular, and I should have found at least ten times the CO_2 evolved. The amount of alcohol formed would also have corresponded to the weight of sugar lost. The extraction of the Yeast has in all my experiments led to the presence of a measurable quantity of alcohol in the digestions at the outset. The Yeast-extract which I obtained from the Tottenham Yeast contained ·4°/₀ of spirit. In no case did I find evidence that this initial amount of alcohol was increased.

My own results receive confirmation from some researches published quite recently by Will (4), and by Lindner (5), Delbruck, and others, who have also failed to extract the enzyme by the method detailed by Buchner.

I do not think that Buchner's statement that the activity of the enzyme is correlated with the presence of coagulable proteid in the Yeast-extract is correct. Certainly I found many extracts containing quite a considerable amount of coagulable proteid to be entirely without enzyme-action when digested with saccharine solutions.

I made some experiments to ascertain whether I could confirm Buchner's statement that the Yeast-cells will, when dry, retain their activity after being heated for several hours to 100° C. I carefully dried the Yeast by exposure to air, after pressing out the Yeast-liquor in a screw-press. When quite mealy to the fingers, I placed a quantity in a beaker *in vacuo* over strong sulphuric acid, and kept it there three days. I then treated half of it to 100° C. for six hours, according to Buchner's directions. On digesting the two quantities with beer-wort at 23° C., there was a copious fermentation at once set up by the unheated sample, but the heated one had lost all power of inducing it.

For the present, therefore, I must contend, in opposition to Buchner, that at any rate our English Yeasts do not contain any alcohol-producing enzyme.

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(2) „ „ „ „ „ 30, 1110–1113.
(3) BLACKMAN: Phil. Trans., 1896.
(4) WILL: Zeits. f. d. Ges. Brauw., 1897, p. 363.
(5) LINDNER: Wochenschr. f. Brau., 1897, p. 364.

The Proteolytic Enzyme of Nepenthes.

BY

S. H. VINES.

THE first indication of the possession by the liquid secreted by the pitchers of *Nepenthes* of the power of digesting proteids was afforded by the observations of Sir Joseph Hooker[1], who found that 'after twenty-four hours' immersion the edges of cubes of white of egg are eaten away and the surfaces gelatinized; fragments of meat are rapidly reduced; and pieces of fibrin weighing several grains dissolve and totally disappear in two or three days.' He does not, however, go so far as to refer the digestive action altogether to the secreted liquid: on the contrary, he says, 'that this process, which is comparable to digestion, is not wholly due to the fluid secreted by the glands, appears to me most probable; for I find that very little action takes place in any of the substances placed in the fluid drawn from pitchers and put into glass tubes.' At the same time he does not state what other agency he assumes to have been operative in the process of digestion or disintegration, though it would appear from the context that probably he had the action of Bacteria in view. It is important to note that he distinctly recognizes an antiseptic action of the liquid, and that he draws attention to changes taking place in the glands

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[1] Address, Brit. Association, 1874; see also Nature, X.

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during the process; 'not only is there aggregation of the protoplasm in the gland-cells, but the walls of the cells themselves become discoloured, and the glandular surface of the pitcher that at first was of a uniform green, becomes covered with innumerable brown specks which are the discoloured glands.'

The publication of Sir Joseph Hooker's observations was quickly followed by more detailed investigations into the nature both of the liquid in the pitchers of *Nepenthes* and of the presumed process of digestion. In 1875 Lawson Tait[1] announced the preparation of 'a substance closely resembling pepsine'—which he describes as 'a greyish flocculent matter'—from the liquid, thus doing for *Nepenthes* what he had already done for *Drosera*. In 1876 von Gorup-Besanez[2] gave an account of the chemical action of the liquid taken from pitchers, some of which had and some of which had not been previously stimulated by the presence of insects in them. He found (1) that the liquid from stimulated pitchers was distinctly acid, whilst that from unstimulated pitchers was neutral or only faintly acid, an observation which agrees with those of Lawson Tait: (2) that the neutral liquid from unstimulated pitchers had no digestive action upon swollen fibrin, even when the experiment was protracted (to twenty-four hours) and the temperature was as high as 20–30° C., whereas the acid liquid from stimulated pitchers digested shreds of fibrin within two hours at a temperature of 20° C., and within one hour at a temperature of 40° C.: and (3) that the addition of a few drops of dilute hydrochloric acid to either the acid or the neutral liquid accelerated the solution of the fibrin in a high degree, so that in one case it entirely disappeared in a quarter of an hour. With regard to the products of the action upon fibrin, he found that the resultant liquid gave no precipitate on the addition of mineral acids, nor with ferrocyanide of potassium after the addition of acetic acid; but gave one with mercuric chloride, tannic acid, and phosphotungstic acid: moreover, with saturated solution of sodium hydrate and dilute solution of copper sulphate it gave a striking rose coloration (biuret-reaction). These reactions indicate the presence of peptone in the liquid, and demonstrate that the fibrin has undergone peptic digestion.

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[1] Nature, XII, 1875, p. 251.

[2] Sitzber. d. phys.-med. Soc. zu Erlangen, 1876.

In the following year[1] I showed that it is possible to obtain a glycerin-extract of the pitchers which has a distinct digestive action on fibrin, though it acts less rapidly than does the pitcher-liquid, thus establishing an analogy between the pitchers and the gastric mucous membrane of animals. I carried this analogy a step further by experiments tending to show that the enzyme of the pitchers, like the pepsin of the stomach, is derived from a zymogen formed in the gland-cells and subsequently decomposed by the action of acids.

It might be fairly inferred that the foregoing observations together form a body of evidence sufficient to warrant the conclusion that the pitchers of *Nepenthes* secrete a proteolytic enzyme; and such was, in fact, the conclusion which was drawn. But of late years the correctness of this conclusion has been questioned. Thus Dubois[2], experimenting with the liquid from the pitchers of various species of *Nepenthes*, arrived at the following results, which I give in his own words:—'Le liquide des urnes fermées, sur le point de s'ouvrir, puisé au moyen d'une pipette à boule stérilisée et avec toutes les précautions convenables pour éviter l'introduction de germes venus du dehors, est resté limpide pendant plusieurs mois. Ce liquide, aussitôt après sa sortie de l'urne fermée, mis en contact avec des cubes d'albumine coagulée, n'a pas attaqué les cubes, ni à la température du milieu ambiant, ni à celle de l'étuve chauffée à 35-40° C.; le liquide resté limpide, filtré au bout de plusieurs heures, ne contenait pas de peptone.'

'La même expérience répétée en puisant le liquide dans les urnes fermées, directement au moyen des tubes à culture de M. Pasteur renfermant des cubes d'albumine, a donné des résultats identiques: les angles des cubes sont restés absolument intacts. Le liquide, examiné au bout de plusieurs jours, ne renfermait pas de microorganismes et aucune trace de putréfaction.'

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[1] Journ. Linn. Soc., XV, 1877, p. 427.

[2] Sur le prétendu pouvoir digestif du liquide de l'urne des Népenthes; Comptes Rendus, t. cxi, 1890, p. 315.

'Le liquide puisé dans les urnes ouvertes depuis très peu de temps, étant encore clair, a attaqué au contraire assez rapidement à la température ordinaire, et très rapidement à la température de l'étuve, les cubes de blanc d'œuf, qui se sont gonflés, sont devenus transparents, gélatineux, et ont perdu leurs angles; le liquide est devenu louche et, dans quelques tubes, il s'est développé une odeur de putréfaction manifeste. Le liquide louche renfermait de nombreux microorganismes de nature diverse et, après filtration, il nous a donné quelques-unes des réactions des peptones.'

These are the observations upon which Dubois relies. He explains his omission to use fibrin as follows:—'Nous nous sommes abstenus de nous servir de fibrine fraîche, parcequ'elle se dissout dans certaines liqueurs acides sans qu'il y ait là une véritable digestion, et qu'elle aurait été cuite pendant la stérilisation: nous avons aussi évité l'emploi de cartilage qui aurait, dans l'autoclave, été en partie transformé en gélatine.'

The following are the conclusions which he draws:—'La manière dont se comporte l'albumine cuite en présence du liquide des urnes de Népenthes, souillé ou non de microorganismes, nous permet de conclure:

'(1) Que ce liquide ne renferme aucun suc digestif comparable à la pepsine, et que les Népenthes ne sont pas des plantes carnivores:

'(2) Que les phénomènes de désagrégation ou de fausse digestion observés par M. Hooker étaient dus, sans aucun doute, à l'activité des microorganismes venus du dehors et non à une sécrétion de la plante.'

Another writer in the same strain is Tischutkin[1]. Unfortunately I am unable to refer to his original paper as it is written in Russian, and have therefore to fall back upon the abstract given in the Botanisches Centralblatt. He denies the secretion of a peptic ferment by any 'insectivorous' plant, supporting his contention by experiments with various plants of this class, including *Nepenthes*, on which he made the following observations, which I quote from the abstract:—'Openings were made in the lateral walls of unopened pitchers, which therefore could contain no Bacteria, and liquid was drawn off by means of a pipette and placed in test-tubes which contained water (neutral in some, acid in others) and cubes of white of egg, the operation being performed throughout under antiseptic conditions. The result was negative; after forty-eight hours at a temperature of 37·5° C., the cubes were unaltered: hence the pitcher-liquid contains no peptic enzyme. In order to anticipate the objection that the pitchers used may have been too young, the experiment was repeated in a modified form. Sterilized cubes of white of egg were introduced into closed pitchers through openings made in their walls, the openings being immediately closed up, and the plants left to themselves. When the pitchers opened themselves four days later, the cubes of white of egg were found to be unaltered; the liquid had a strong acid reaction, but it contained no peptone, and but very few Bacteria were present. The liquid from these pitchers was then placed in test-tubes with fresh cubes of white of egg; the cubes underwent solution in four or five days, that is, after a time which permitted the Bacteria to multiply to a very considerable extent.'

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[1] Ueb. die Rolle der Mikroorganismen bei der Ernährung der insectfressenden Pflanzen, Arb. d. St. Petersburger Naturf.-Gesellschaft, 1891; Bot. Centralblatt, 50, 1892, p. 304.

The conclusions of Dubois and of Tischutkin have been adversely criticized by Goebel[1], who adduces evidence favouring the view of the presence of a proteolytic enzyme in the secretion of *Nepenthes*. For instance, he made the following observations on three pitchers of *N. paradisiaca* of different ages. The oldest pitcher contained a small quantity of neutral liquid; a wasp which made its way into it soon died, and the liquid acquired an alkaline reaction, many Bacteria and Infusoria being present in it. The second pitcher contained acid liquid, in which there was a fly: the liquid dissolved fibrin in an hour, and after three hours it contained no dissolved albumen, but only peptone; another shred of fibrin was dissolved by the liquid in forty minutes at a temperature of 16–18° C., when some 0·2 $^{\circ}/_{0}$ solution of hydrochloric acid had been added to it; a gelatin-culture from this liquid gave no indication of the presence of Bacteria. The youngest pitcher was still closed: it contained 4·6 c.cm. of a neutral viscid liquid: a gelatin-culture from the liquid gave no indication of the presence of any Fungi; on the addition of formic acid to the extent of ·001, a shred of swollen fibrin was dissolved by it in twelve hours. Goebel did not succeed in preparing an active glycerin-extract of the pitchers. He concludes that these facts suffice to invalidate the assumption that the digestive process which goes on in the pitchers is to be attributed to the action of Bacteria, which only comes into play in the case of old and exhausted pitchers, the liquid in which has no longer an acid reaction.

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[1] Pflanzenbiologische Schilderungen, II, 1893, p. 186.

In view of Goebel's observations, it may perhaps seem superfluous to reopen the subject: but inasmuch as the statements of Dubois and of Tischutkin are in direct contradiction to my own published results, I have felt it necessary to repeat my experiments, and to extend and modify them in various directions. I hope that the new facts which I am now able to bring forward may be accepted as my justification.

Goebel has already commented upon the insufficiency of Dubois' data as a basis for the sweeping conclusions which he draws: but there is one point to which I would draw special attention. One of the most important pieces of evidence upon which Dubois relies is the fact that liquid drawn directly from unopened pitchers exerted no digestive action upon cubes of white of egg. It is to be remarked that he himself describes the liquid in unopened pitchers as 'légèrement acide.' Now, no one has ever asserted that liquid from unopened pitchers will digest cubes of white of egg; it would be as reasonable to expect an extract of gastric mucous membrane to digest in the absence of an adequate supply of acid. Had he added a few drops of ·2% HCl to the liquid, I feel certain that his results would have been altogether different; this is, in fact, an experiment which ought to have been made before he allowed himself to form any definite opinion.

Tischutkin's observations certainly appear to be more formidable than those of Dubois. It is impossible for me to attempt any adequate criticism of them, as the original paper is inaccessible to me, and the abstract in the Bot. Centralblatt does not give the details of the experiment with sufficient minuteness. It would appear, however, that Goebel's observations counterbalance them; and to his I would add the weight of the following of my own.

ACTIVITY OF PITCHER-LIQUID.

In these experiments the material used for digestion was chiefly well-washed blood-fibrin preserved in a mixture of one part pure glycerin and two parts distilled water: coagulated egg-albumen was used but rarely, inasmuch as it is much more resistent to digestion than is fibrin, and the action is relatively slow. The fibrin preserved in glycerin may be regarded as free from Bacteria, in view of the results obtained by Copeman[1] with regard to the antiseptic action of glycerin.

As regards Dubois' objection to the use of fibrin in these digestive experiments, it is, I think, sufficiently met by the fact that I have frequently made control-experiments with tubes containing only dilute HCl and fibrin, but no pitcher-liquid, and have invariably found that by the time that the fibrin in the tubes containing the pitcher-liquid had completely disappeared, that in the control-tubes, though gelatinous, showed no signs of breaking up or of solution.

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[1] Copeman and Blaxall, Experiments on the Action of Glycerine upon the Growth of Bacteria; Brit. Assoc. Report, 1896.

The pitcher-liquid used was obtained from *N. Mastersiana*, and I will begin the account of my observations by saying that in all the very numerous experiments which I have made, I have never once failed to obtain digestion of fibrin by the pitcher-liquid, provided that an adequate amount of acid, which I supplied in the form of a ·25% solution of HCl, was present. I give the details of one experiment in illustration.

Nov. 13, 1896. Two test-tubes were prepared, each containing 5 c.cm. of neutral pitcher-liquid, and a shred of fibrin: to the one (*A*) 5 c.cm. of ·25% HCl were added, to the other (*B*) 5 c.cm. of distilled water: the tubes were placed in the incubator (about 35° C.) at 11.30 a.m. At 2.30 p.m. the fibrin in tube *A* was completely dissolved, the liquid giving a good biuret-reaction. The fibrin in tube *B* was still undissolved at 9 a.m. on the following morning: 5 c.cm. of ·25% HCl were then added to it, with the result that the fibrin had entirely disappeared by 11.30 a.m.

In a similar experiment to the foregoing, made on May 7, 1897, with distinctly acid liquid taken direct from unopened pitchers, the fibrin in the tube (*A*) to which the HCl had been added, dissolved within half an hour; whilst that in the other tube (*B*) showed no change after several hours.

It is unnecessary to insist further upon the fact that the pitcher-liquid exerts an unmistakable solvent action on fibrin: the crucial point is whether or not it exerts this action under conditions such as to render highly improbable the assumption that the action in question is due to Bacteria rather than to a proteolytic enzyme. I have endeavoured to determine this point by making digestion-experiments in the presence of recognized antiseptics, with the following results:—

Dec. 2, 1896. Prepared three test-tubes as follows: each tube contained some pitcher-liquid acidified with ·25% HCl, and a shred of fibrin: to (1) was added some potassium cyanide; to (2) some thymol; to (3) a few drops of chloroform: at 1 p.m. the tubes were placed in the incubator at 35° C. The fibrin in tube (1) was completely dissolved by 5 p.m.; that in the other two tubes was broken up by that time, and was found to have entirely dissolved by the following morning at 9 a.m.

Another experiment of the same kind was the following. Six tubes were prepared, each containing 10 c.cm. of 0·25°/$_0$ HCl and 5 c.cm. of pitcher-liquid: to each of three of the tubes (*A*, *B*, *C*) a fragment of coagulated egg-albumen was added, and to each of the other three (*D*, *E*, *F*) a shred of fibrin: to tubes *A* and *E* some KCy (about 0·05 grm.) was added; to tubes *C* and *F* some thymol; to tubes *A* and *D*, nothing. The tubes were all placed in the incubator, temp. 35° C., at 4.30 p.m. on Jan. 25, 1897, and left all night. On the following day it was observed at 9 a.m. that the albumen in tubes *A* and *C* had undergone partial solution, whilst that in *B* had been acted upon in a less degree; at 3 p.m. the albumen in *A* was nearly all dissolved, that in tube *C* less so, that in tube *B* still less: all these liquids gave the biuret-reaction, though it was faint in *B*. The fibrin-tubes showed, at 9 a.m., complete solution in tube *D* and in tube *F*, and nearly complete solution in tube *E*.

More recently I have experimented with corrosive sublimate ($HgCl_2$) with satisfactory results.

Oct. 1, 1897. Two test-tubes (*A* and *B*) were prepared as follows: each contained, to begin with, 10 c.cm. of pitcher-liquid, 5 c.cm. of 0·25°/$_0$ HCl solution, and a shred of fibrin: to *A* was added 5 c.cm. of a 1°/$_0$ solution of $HgCl_2$, and to *B* 5 c.cm. of distilled water. The tubes were placed in the incubator at 11 a.m.: at 12.30 p.m. the fibrin in *B* was completely dissolved; that in *A* was completely dissolved at 3 p.m.

Another similar experiment was made with two tubes, to each of which a definite equal weight of fibrin ($\frac{1}{10}$ grm.) was added: the one tube *Y* contained the $HgCl_2$, the other tube *Z* contained none. The two tubes were placed in the incubator at 1 p.m.: at 2 p.m. the fibrin in tube *Z* was completely dissolved, whereas that in tube *Y* was not completely dissolved until 3.30 p.m.

After having performed the foregoing experiments with antiseptics, I happened to come across Fiechter's [1] investigations into the action of hydrocyanic acid (HCN) on organized ferments and enzymes, from which it appears that whilst the acid in minute quantity arrests the action of, and even kills, Yeasts and Bacteria, it has little or no effect upon the action of enzymes unless present in large proportion. I accordingly arranged the following experiment:—

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[1] Fiechter, Ueb. den Einfluss der Blausäure auf Fermentvorgänge, Basel, 1875.

Oct. 27, 1897. 5 c.cm. of pitcher-liquid were placed in each of two tubes *A* and *B*. To *A* were added 5 c.cm. of $2^{\circ}/_{0}$ HCN solution, and one drop of strong HCl: to *B* were added 5 c.cm. of a $0{\cdot}5^{\circ}/_{0}$ solution of HCl, and to each 0·05 grm. of fibrin. The tubes were placed in the incubator (37° C.) at 11 a.m.: at 12.30 p.m. the fibrin had completely disappeared in both tubes.

This experiment appears to me to absolutely exclude the hypothesis of bacterial action, and to establish beyond doubt the fact that the pitcher-liquid of *Nepenthes* contains a proteolytic enzyme.

However, I felt it advisable to secure my position by experiments on the other side; by experiments of such a kind that whilst the conditions were favourable to bacterial action, they should be fatal to the action of an enzyme, and that, under these conditions, no solution of fibrin should take place. It is known that whilst alkalies are destructive of enzymes, they are not nearly so fatal to organized ferments. My experiments were then directed to determine the effect of treatment with alkalies upon the digestive activity of the pitcher-liquid. The general plan of the experiments was to treat some of the liquid for a time, at a temperature of 35°–40°C., with alkalies in various strengths; and then, after neutralizing the liquid, to test its digestive activity in the usual way. Without going into further detail, I may briefly state that the alkalies which I used were sodium carbonate ($Na_2 Co_3$) and sodium hydrate (Na HO), and that I find the activity of the liquid to be destroyed by treatment with $1^{\circ}/_{0}$ Na HO for one hour, and by treatment with $5^{\circ}/_{0}$ $Na_2 CO_3$ for three hours. I hope to determine these limits more definitely in the future. I may incidentally mention that the addition of these alkalies produced, in every case, a precipitate in the pitcher-liquid; but I have not yet been able to ascertain the nature of this precipitate.

The investigation of the action of alkalies upon the activity of the enzyme, led me on to study the relation of its activity to the amount of free acid present in the digesting liquid. For this purpose I prepared four tubes, *A*, *B*, *C*, *D*, containing respectively $1^{\circ}/_{\circ}$, $0{\cdot}5^{\circ}/_{\circ}$, $0{\cdot}25^{\circ}/_{\circ}$, and $0{\cdot}125^{\circ}/_{\circ}$, of HCl, and set them in the incubator to digest. I found that digestion was most rapid in tube *C*, containing $0{\cdot}25^{\circ}/_{\circ}$ HCl, 0·05 grm. of fibrin totally disappearing in about half an hour; the fibrin disappeared in tube *D* in about a quarter of an hour more; that in tube *B*, about twenty minutes later; whereas in tube *A*, which contained the largest percentage of acid, the fibrin had not disappeared two hours subsequently.

Another line of experimentation which I have pursued is that of obtaining from the pitcher-liquid a substance which would exert digestive power. I have not attempted to actually isolate the proteolytic enzyme, but only to remove it from the pitcher-liquid in such a form that a new digestive liquid could be prepared with it. I have repeated this experiment several times, and with unvarying success. The method adopted is illustrated by the following experiment:—

Sept. 21, 1897. To 100 c.cm. of pitcher-liquid an equal volume of absolute alcohol was added: then, in order to produce a bulky precipitate in the liquid, some phosphoric acid was added and some lime-water, the liquid being finally neutralized with carbonate of ammonia: a copious precipitate fell, which was collected on a filter and left to drain all night.

Sept. 22. A portion of the precipitate was shaken up with 10 c.cm. of a $0{\cdot}25^{\circ}/_{0}$ solution of HCl, and the turbid liquid filtered: to the clear filtrate a small piece of fibrin was added, and the tube (*A*) was placed in the incubator at 11 a.m. A second tube (*B*) was prepared, containing 10 c.cm. of $0{\cdot}25^{\circ}/_{0}$ HCl and a shred of fibrin; this was placed in the incubator at the same time as tube *A*. At 1 p.m. the fibrin in tube *A* was breaking up, and at 2 p.m. it had entirely disappeared, the liquid giving good biuret-reaction: the fibrin in the control-tube *B* showed no sign of solution.

On repeating the experiment a month later with some of the precipitate which had been kept dry in a bottle in the presence of chloroform-vapour, I obtained complete solution of 0·05 grm. of fibrin in three hours.

CHEMISTRY OF THE PITCHER-LIQUID.

Our knowledge on this point is very incomplete. So far as I am aware, the only chemical examinations of it which have been made are, first, that recorded in the Botanical Magazine for 1828, and, secondly, the analysis made by Voelcker in 1849. The former account states that the liquid in the unopened pitcher of *Nepenthes distillatoria* 'had a sub-acid taste, which increased after the rising of the lid, when the fluid slowly evaporated. My friend Dr. Turner perceived it to emit, while boiling, an odour like baked apples, from containing a trace of vegetable matter, and he found it to yield minute crystals of superoxalate of potash on being evaporated to dryness.' Voelcker[1] found that the liquid from unopened pitchers of species of *Nepenthes* (names omitted) gave a dry residue which varied from 0·27 to 0·92 °/₀, of which about two-thirds consisted of incombustible ash; he estimates its percentage composition as follows:—

Malic and citric acids	38·61
Potassium chloride	50·42
Soda	6·36
Lime	2·59
Magnesia	2·59

The liquid obtained from the pitchers may be generally described as slightly opalescent, colourless, and somewhat viscid, especially when drawn from unopened pitchers. Its reaction varies from neutral to acid, but the conditions of this variation are undetermined. Hooker states that the liquid is always acid, whilst von Gorup-Besanez describes the liquid from 'unstimulated' glands as neutral or very slightly acid, whilst that from 'stimulated' glands (*N. Phyllamphora* and *N. gracilis*) was distinctly acid. Again, Goebel found the liquid in unopened pitchers of *N. paradisaica* to be neutral, but that in those of *N. Mastersiana* to be acid. As the result of my own observations on *N. Mastersiana*, I find that the liquid in the unopened pitcher is generally distinctly acid, though not always so; and that the liquid in the opened pitchers, in which the remains of insects may be present, is as often neutral as it is acid. The correlation suggested by von Gorup-Besanez between the reaction of the liquid and the 'stimulated' or 'unstimulated' condition of the glands, does not, therefore, appear to hold good.

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[1] Voelcker, Ann. and Mag. of Nat. Hist., IV, 1849, p. 128.

I have examined the liquid with a view to determine its proteid content, with the result that I have found it to contain only a minute quantity. Liquid which digests actively, and even when containing the débris of insects, gives, after filtration, only a very faint xanthoproteic reaction, no precipitate with nitric acid, with potassium ferrocyanide and acetic acid, or with platinic chloride, nor any turbidity on boiling: it gives a precipitate with Millon's reagent which, on heating, does not turn red but yellow.

In view of the fact that the liquid is quite inert in the absence of acid, it occurred to me that possibly it might contain the enzyme in the form of a zymogen. In order to assure myself on this point, I had recourse to the method described by Langley and Edkins[1], which is based on the fact that, if a current of CO_2 be passed through a liquid containing both zymogen and enzyme, the zymogen is more rapidly destroyed than the enzyme, a method which was employed by Green in his researches on germinating seeds. The experiment was as follows:—

Oct. 16, 1897. Two tubes, each containing 75 c.cm. of neutral liquid from opened pitchers of *N. Mastersiana*, were taken: to the one, tube *A*, were added 25 c.cm. of a 0·5°/₀ solution of HCl, so that the liquid contained 0·125°/₀ of the acid, and the tube was then warmed for an hour in the incubator at 37° C., when it was removed from the incubator, and the liquid was neutralized with 15 c.cm. of a 1°/₀ solution of Na_2CO_3. To the tube *B* was now added a mixture of 25 c.cm. of 0·5°/₀ solution of HCl and 15 c.cm. of the 1°/₀ solution of Na_2CO_3. The amount of liquid in each case was now 115 c.cm., and the amount of salts in each was identical. CO_2 was then passed through both liquids for an hour: then 15 c.cm. of each were placed in a tube, 10 c.cm. of 0·5°/₀ HCl being added to each, together with 0·1 grm. of fibrin, and the tubes were placed in the incubator to digest at 1 p.m. At 3 p.m. the fibrin in both tubes was entirely dissolved, showing that their activity was the same.

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[1] Langley and Edkins, Journal of Physiology, VII, 1886; Green, Phil. Trans. Roy. Soc., 178 B, 1887.

On repeating the experiment, prolonging the treatment with CO_2 to an hour and a half, I obtained the same result; whence I conclude that the liquid contains no zymogen.

The general conclusion at which I arrive is that either the enzyme is not a proteid, or, if it is, it is present in extremely minute quantity, though it is difficult to accept this alternative in view of the remarkable digestive activity of the liquid.

ACTIVITY OF GLYCERIN-EXTRACTS.

I have repeatedly confirmed my observation of 1877 that active glycerin-extracts of the pitchers can be prepared: in but few cases have I found these extracts, when fresh, inoperative on fibrin, though they acted much less rapidly, as might be expected, than the pitcher-liquid. In my experiments of 1877, and in the earlier ones of this series, the preparation of the extract was preceded by treatment of the pitchers for some hours with absolute alcohol. But recently I have found that a more active extract can be prepared by treating the pitchers, finely cut up, at once with pure glycerin, omitting the treatment with absolute alcohol. The glycerin is left in contact with the pitcher-material for at least a week before filtering off and using for an experiment. The pitchers used were in all cases vigorous, either opened or unopened. The following experiment affords some idea of the relative activity of two glycerin-extracts, the one prepared with, the other without, previous treatment of the pitcher-material with absolute alcohol:—

Sept. 3, 1897. Three unopened pitchers of *N. Mastersiana* were removed from the plant: the upper third of each pitcher was cut off, the contained liquid poured off, and the pitchers, after well washing with water and drying, were chopped up fine. Of this material 4 grms. were placed in absolute alcohol for 24 hours; then rubbed up in a mortar with 40 c.cm. of pure glycerin and let stand (extract *A*): other 4 grms. were at once rubbed up in a mortar with 40 c.cm. of pure glycerin (extract *B*).

On Sept. 13 both extracts were filtered through muslin to remove the coarser particles of pitcher-substance.

On Sept. 14 the following experiment was made: 2·5 c.cm. of extract *A* were placed in a test-tube with 5 c.cm. of 0·25°/$_0$ of HCl and a shred of fibrin; similarly, the same quantity of extract *B* was placed with the same quantity of 0·25°/$_0$ HCl and a shred of fibrin, in another test-tube: the two tubes were placed in the incubator at 11.45 a.m. At 1 p.m. the fibrin in *B* showed signs of breaking up, whereas this was not observed in *A* until 2 p.m.: at 4.30 p.m. the fibrin in *B* had entirely disappeared, whilst some still remained in *A* at 5 p.m. but had altogether disappeared by the following morning.

On Oct. 4, 1897, a similar comparative experiment was made with the same extracts, an exactly equal amount ($\frac{1}{10}$ grm.) of fibrin being placed in each tube at 11 a.m.: the fibrin was entirely dissolved in tube *B* at 4 p.m., whereas in tube *A* it had not disappeared by 5 p.m., and did not disappear after being left in the unheated incubator all night, until it had been heated for an hour (9–10 a.m.) on the following morning.

The glycerin-extracts do not retain their activity for a prolonged period, though I am not able to say exactly for how long. For instance, in an experiment made on October 4, 1897, with extracts prepared on December 2, 1896, the fibrin showed no change after forty-eight hours. In experiments made on October 6, 1897, with extracts prepared respectively on May 7 and May 20, the fibrin underwent but little change in twenty-four hours. The extracts in question had been found to be active when fresh, digesting in a few hours. However, I have found an extract two months old to be active.

I have not found that an active glycerin-extract can always be prepared from pitchers. It appears that this can only be done with relatively young pitchers; and it is probable that the secretion of the enzyme ceases some time before the pitcher shows signs of withering. I may add that I have found glycerin-extract to act upon fibrin in presence of 1°/₀ HCN, though the digestion is slightly retarded.

I attach considerable importance to the activity of glycerin-extracts of the pitchers, because it proves the existence of a digesting agent in their tissue, probably in the glands, and thus affords collateral evidence that this agent is not a Bacterium but an enzyme. It is highly improbable that any Bacterium would retain its vitality after two months sojourn in pure glycerin.

Moreover, the fact that an enzyme can thus be extracted, suggests that a zymogen must be present. In the course of the last eighteen months, I have several times endeavoured to repeat my observation of 1877, which revealed the presence of a zymogen, but so far, I must admit, without success. On the contrary, I have generally found that previous treatment with acid rather diminishes than increases the activity of the resulting extract. I propose to make this point the object of further research next season; my main object in the observations recorded in this paper having been the refutation of the suggestion that the digestive activity of the pitcher-liquid is due to Bacteria.

THE PRODUCTS OF DIGESTION.

Our knowledge of the products of the digestion of fibrin by the pitcher-liquid is derived entirely from the observations of von Gorup-Besanez, who describes them as peptones. He found, namely, that the filtered liquid after digestion remained clear on boiling, and gave no precipitate with mineral acids, nor with potassium ferrocyanide in the presence of acetic acid; whilst it gave a precipitate with corrosive sublimate, tannic acid, or phospho-tungstic acid, as also a brilliant biuret-reaction.

My observations lead me to results differing widely from those of von Gorup-Besanez. I find, in the first place, that the digested liquid always gives a precipitate with nitric acid, giving a clear yellow liquid on warming which again becomes turbid on cooling, as also a precipitate with potassium ferrocyanide and acetic acid: the biuret reaction is well marked. On neutralization, there is a precipitate, dense when the digestion has been of short duration (two hours), which consists presumably of parapeptone. After filtration from this precipitate, the neutral liquid gives only a slight turbidity on the addition of nitric acid, though it contains much proteid: hence the substance precipitated by nitric acid is probably some parapeptone not precipitated on neutralization.

On concentrating the neutral liquid on the water-bath to one-third or one-fourth of its bulk, and then filtering into alcohol, a considerable precipitate is formed. The alcohol having been removed by filtration, the precipitate on the filter mostly dissolves in a small quantity of distilled water, the solution giving the following reactions: (1) no precipitate with nitric acid, but a strong xanthoproteic reaction; (2) a precipitate with potassium ferrocyanide and acetic acid, (3) a striking biuret-reaction; (4) a dense precipitate on saturation with ammonium sulphate, the filtrate from which gives no proteid reactions. From this I conclude that the main product of digestion is a proteid of the nature of an albumose, allied, by its not being precipitated with nitric acid, to the body known as deutero-albumose. I failed to detect the presence of a true peptone, that is, of a proteid which is not precipitated on saturation with ammonium sulphate.

I have tested the dialysing properties of the proteid precipitated by alcohol, and find that after twenty-four hours the water outside the dialyser gives a slight xanthoproteic reaction, but no biuret, though the liquid in the dialyser gives both reactions very strongly, whence I conclude that there is no readily dialysable proteid present.

In most cases the investigation of the products of digestion was proceeded with immediately after digestion: but in one case I kept the proteids precipitated by alcohol, in alcohol, for nearly a year, without finding their solubility in water or their reactions in any way altered. The duration of the digestion was varied in different cases, from as short a time as two hours, to as long a time as six days; the only perceptible difference in the nature of the products being that in the case of the brief digestions a much larger quantity of parapeptone was present. In some of the digestions I have used as much as ten grammes of fibrin; more often five or six grammes: the digestive liquid usually contained about 0·2°/$_0$ HCl, and the amount of liquid was about 20 c.cm. to each gramme of fibrin, of which nearly half consisted of pitcher-liquid.

I have endeavoured to determine the nature of the ultimate products of digestion, but without complete success. I find that if the digested liquid be neutralized and be allowed to stand for some hours, a deposit gradually collects on the walls of the beaker. Under the microscope this substance is seen to consist of minute round granules, cohering in rows and in clumps, or of vacuolated oily drops. On carefully evaporating some of this with nitric acid, and then adding a drop of caustic soda, a deep brown colour is produced (Scherer's test for leucin). I have obtained apparently the same substance from the precipitate formed when the digested liquid is dropped into alcohol. The dense precipitate is not wholly soluble in cold distilled water; and when hot water is poured over the insoluble residue on the filter a turbid liquid passes through. This liquid becomes clear on heating, but reprecipitates on cooling; it also becomes clear on the addition of acids and alkalies, and gives the foregoing colour-reaction on treatment with nitric acid and caustic soda. It gives a faint yellow colour on the addition of ammonia, after boiling with nitric acid; and a precipitate with Millon's reagent, which turns partly red and partly yellow on heating: on adding caustic soda in excess and a small quantity of solution of copper sulphate there is no precipitate of copper hydrate, nor is there any biuret-reaction. I am inclined to infer that this substance is one of the ultimate products of digestion, as also to conclude that it may be leucin or some allied body: but I have failed to find the characteristic scales of leucin in sufficient quantity. I propose to continue investigation in this direction.

I may add that I have tried the action of the pitcher-liquid upon the gluten of wheat, which it readily digests, and that I have found the products of digestion to be essentially the same as those obtained by the digestion of fibrin.

CONCLUSION.

The fact that the pitcher-liquid digests fibrin in the presence of 1% hydrocyanic acid, together with the fact that it is possible to prepare active glycerin-extracts from the pitcher-tissue, seems to me to conclusively prove that the proteolytic digestion is due to an enzyme and not to a Bacterium. I would go so far as to urge that the *onus probandi* is now transferred to those who maintain that the process is one of bacterial action. Before this view can be accepted, it is incumbent upon those who support it to produce an organism which will digest fibrin in the presence of 1% of hydrocyanic acid, and which will retain its digestive activity when kept for several weeks in pure glycerin. Until this is done there can be no valid argument against the enzyme-theory.

With regard to the nature of the enzyme, whilst it is clearly allied to the peptic group inasmuch as it is only active in an acid medium, it is apparently tryptic in its action, like all the other better known proteolytic enzymes of plants, with no one of which it can, however, be identified. So far as I am aware, the following are the only cases in which the products of proteolytic vegetable enzymes have been determined with any accuracy. Von Gorup-Besanez states that the peptic enzyme which he extracted from Vetch-seeds[1] dissolved fibrin with the formation of peptone, and he makes the same statement with regard to the enzyme of *Nepenthes*[1]: but he did not specially investigate the products of digestion. With regard to papain, which is, however, an altogether tryptic enzyme, active in neutral or alkaline liquids, Martin states that it produces peptone, leucin and tyrosin from fibrin[2], though it does not produce true peptone from vegetable proteids[3], but a 'peptone-like body' which splits up into leucin and tyrosin. Finally Green[4] has found that the proteolytic enzyme in the germinating seeds of *Lupinus hirsutus* produces, both from fibrin and vegetable proteids, a 'so-called peptone,' leucin, and tyrosin; and though it is only active in an acid medium, he regards the enzyme as 'tryptic rather than peptic.' The assertion that peptone is a product of the activity of these enzymes is based, by von Gorup-Besanez, on the reactions already enumerated (p. 578); and by Martin and Green mainly on the results of dialysis. It must, however, be pointed out that neither the reactions nor the dialysis are absolutely conclusive on the point. The only certain proof that a liquid contains true peptones lies in the fact that such a liquid continues to give proteid reactions after saturation with ammonium sulphate, since this neutral salt precipitates all proteids except true peptones: but this test was not applied in any of the above-mentioned researches. As I have already pointed out, I have invariably found that a solution of the products of digestion by pitcher-liquid gives no proteid reactions after saturation with ammonium sulphate, all the proteids present having been precipitated by the salt. I conclude, therefore, that true peptone is not one of the products of digestion. The proteid product of digestion appears to be deutero-albumose. It is, however, possible that true peptone may be formed, and be immediately split up into leucin or bodies of that kind. Otherwise it must be assumed that the albumoses formed in digestion are directly split up into these bodies, a view which gains some support from the fact that

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[1] Von Gorup-Besanez, Sitzber. d. phys.-med. Soc. zu Erlangen, 1874; ibid. 1875.

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[1] Von Gorup-Besanez, Sitzber. d. phys.-med. Soc. zu Erlangen, 1876.

[2] Journal of Physiology, V, 1884.

[3] Ibid. VI, 1885.

[4] Phil. Trans. Roy. Soc., vol. 178, B, 1887.

Martin failed to find peptone among the products of digestion of vegetable proteids by papain, though leucin and tyrosin were formed. However, it does not necessarily follow that because the proteolytic enzyme of *Nepenthes* apparently does not give rise to peptone, no other vegetable enzyme does so. For it must be borne in mind that the enzyme of *Nepenthes* has a function to perform which is quite different to that of the other enzymes under consideration: they are destined to act only on the proteids in the tissues of the plant, whereas the enzyme of *Nepenthes* has to act upon the proteids of animals outside the plant, and to change them into substances which can be readily absorbed. Still there is one feature which all these enzymes undoubtedly possess in common, namely this, that they all produce relatively large quantities of albumoses. The whole subject of the action of vegetable proteolytic enzymes obviously requires careful re-investigation.

Another peculiarity of the enzyme of *Nepenthes* is its great stability. It resists decomposition, and is indeed antiseptic, differing in this respect from papain (and trypsin) which, as Martin points out, is readily decomposed; and its activity is not destroyed by treatment with such a proportion of alkali as Green has found to destroy that of the enzyme of the Lupin-seed. This greater stability is no doubt to be correlated with the fact that it has to act outside the plant, where it may be exposed to conditions which would render it useless were it less stable than it is.

The secretion of the necessary acid by the pitchers is an important point to which I have not been able as yet to give any attention. I would only lay stress upon the fact that, in *N. Mastersiana*, the liquid in the unopened pitchers is, as a rule, distinctly acid, a fact which controverts the prevalent idea that the secretion of acid is the result of stimulation by the presence of foreign bodies in the pitchers. The whole physiology of the glands requires investigation by those histological methods which Miss Huie has so successfully employed in the study of the glands of *Drosera*[1].

[1] Huie, Quarterly Journal of Microscopical Science, 1896.

The material of *N. Mastersiana* which I have used was for the most part obtained from the Oxford Botanic Garden, but I am very much indebted to Messrs. Veitch, and to the authorities of the Royal Gardens, Kew, for further supplies of liquid and pitchers of this and other species.

NOTES.

THE EFFECTS OF TROPICAL INSOLATION.—In my paper with this title, published in the September number of the Annals, I notice that, when speaking of the movements of the sensitive leaflets, I omitted to refer to Oltmann's researches on this subject. Oltmann[1] was the first to show that in *Robinia* it is the pulvini which are directly stimulated to movement by a change in the intensity of the illumination. The observations I have made in Java and Ceylon are of interest as corroborating and extending the results obtained by Oltmann, and later by Macfarlane[2], on this particular point.

ALFRED J. EWART.

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THE TENSILE STRENGTH OF CELL-WALLS.—Some time ago I estimated the osmotic pressure in the leaf-cells of several plants to range between 20–30 atmospheres (or 200–300 grs. per sq. mm.)[3]. It seemed to me of interest to ascertain what coefficient of safety was allowed by the plant to meet these high pressures. This may be done by determining the tenacity or tensile strength of cellulose, and estimating the stress in the cell-wall while the cell is distended by these pressures.

The first was ascertained directly by weighing a single hair or fibre, detached from the surface of the seed of *Gossypium*, until it broke. From the breaking weight and the area of cross-section of the fibre, its tenacity may be obtained.

I applied the weight by slowly filling with water a light paper bucket attached to the fibre. The attachment was effected by gluing the end of the fibre to a fine wire-handle on the bucket.

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[1] Oltmann, Flora, 1892, p. 234.

[2] Macfarlane, Bot. Centralblatt, 1895, I, p. 136.

[3] Notes from the Botanical School, Trinity College, Dublin, No. 2, May, 1897.

To quote an example of one of these observations: the bucket suspended from a fibre, as described, was slowly filled with water. When the increasing weight parted the fibre, the bucket together with the water weighed 7·817 grs. Microscopic measurement showed that the greater diameter of the fibre was 0·0192 mm., and the lesser 0·0078 mm. As the fibres are roughly rectangular in section, the area of cross-section equalled 0·00015 sq. mm. inclusive of the lumen of the cell, which, however, is very small.

From this it follows that the breaking stress was not less than 52,000 grs. per sq. mm.

Another experiment gave the weight of bucket and water sufficient to break another fibre as 8·500 grs. But in this case the cross-section of the fibre was 0·0137 mm. × 0·0168 mm. It follows that the breaking stress was greater than 37,000 grs. per sq. mm.

It is evident from the nature of the method that these figures only fix a minor limit to the tenacity of cellulose. The breaking stress of cellulose must be as great or greater than 52,000 grs. per sq. mm. For it is impossible to determine the cross-section exactly at the point of fracture, and it has been my practice to estimate the section at the narrowest place above the fracture; but of course this is not the smallest cross-section, supposing the fibre to be homogeneous. It seems indeed that the figures are much below the true breaking stress, and that fracture occurs at a point in the fibre weakened by some flaw. This may be demonstrated by taking one of the parts of a fibre, which originally parted under a stress, say, of 3 grs., and testing it in the same manner as before. It will then be found to support a greater weight: probably of 10 grs. or even 12 grs. One fibre, ·017 × ·018 mm., broke on the first trial at 8·500 grs., second at 12·552, third at 16·267, and fourth at 18·200 grs. The final result gives a tenacity of 60,000 grs. per sq. mm.

Hence we may safely assume that the tenacity of cellulose is greater than 50,000 grs. per sq. mm., which is about equivalent to thirty tons per square inch, and also about the same as the tenacity of Aluminium bronze[1].

The tenacity given for Red Pine[2] is 9,100 grs. per sq. mm.; but the actual area of woody wall in a section of Pine, would scarcely amount to more than about 0·2 of the cross-section; so that the absolute tenacity of the substance of the walls of the tracheides probably approximates closely to that of the fibre of *Gossypium*.

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[1] Numerical Tables and Constants, S. Lupton. Macmillan, 1896, p. 17.

[2] Loc. cit.

Now with regard to the stress actually obtaining in the cell-walls of the leaf. It will be convenient, in treating of the stresses, to regard the cells as cylinders. This assumption is justified by the consideration that it is probably only the cell-walls of the cylindrical cells of the leaf, e.g. those of the palisade-tissue and those forming sheath surrounding the bundles, which are exposed to this stress; for it seems probable that the walls of the irregularly stellate cells of the spongy parenchyma are not exposed to any great distending forces, otherwise they would assume a spherical contour; unless, indeed, we attribute great rigidity to their cell-walls. The apparent paradox, that these cells contain a pressure of 20–30 atmospheres, while there is no stress in their walls, is explained by the fact that it is an osmotic pressure, and is not exerted on the enclosing membrane unless there is fluid enough present to distend that membrane. This consideration would also lead to the conclusion that the rigidity of leaves is due only to the cylindrical and more spherical cells, and that those cells with re-entrant angles are not normally in a state of turgor.

When a cylinder is exposed to internal pressure, the stress in the wall tending to rupture it may be obtained in the following way: the total disruptive force equals the internal pressure acting over an area equal to the length multiplied by the diameter of the cylinder. This force is exerted on an area of cellulose equal to the thickness of the cell-wall multiplied by twice the length and twice the diameter of the cylindrical cell. Thus—

$$\text{Stress per sq. mm. of cellulose} = \frac{\text{P. l. d.}}{2\ (\text{l}+\text{d})\ \text{t}.}$$

Where P = osmotic pressure, l = length, d = diameter of cell, and t = thickness of cell-wall.

In *Cytisus Laburnum* the cylindrical palisade-cells are about ·06 mms. long, about ·0175 mms. diameter, and their cell-wall is ·001 mms. thick. The stress per sq. mm. in their cell-walls is consequently

$$\frac{300 \times \cdot 06 \times \cdot 0175}{\cdot 155 \times \cdot 001} = 2{,}032 \text{ grs.}$$

In *Helianthus multiflorus* a cell from the same tissue measures very closely the same; so that in these two instances we see that the walls of the cells of the palisade-parenchyma, so far as they are exposed to disruptive forces, are stressed far below the experimentally derived tenacity of cellulose. In fact there is a coefficient of safety amounting to 25.

The dimensions of the cylindrical cells of the sheath are the following:—

Cytisus Laburnum, length=·100 mm., diameter=·030 mm.

Helianthus multiflorus, length=·108 mm., diameter=·018 mm.

These are the maximum dimensions observed. The thickness of the walls in each case is about ·001 mm.

It results from these figures that if the pressure is 300 grs. per sq. mm. that cell-walls of *Cytisus Laburnum* will be subject to a stress of less than 3,500 grs. per sq. mm. This leaves a coefficient of safety of at least 14.

It has been noticed as probable that the walls of the cells of the spongy parenchyma do not, under normal circumstances, sustain any appreciable stress. But even if it is assumed that their cellulose membrane is sufficiently rigid to maintain its irregular shape when exposed to these high internal pressures, it is easily shown that the stress in the cell-walls does not rise above 1,500 or 2,000 grs. per sq. mm.

So it appears that the wall of every cell of the leaf is amply strong to sustain the high osmotic pressures which are demonstrable as giving rigidity to the leaf, and that, so far as the strength of the cell-walls is concerned, much higher osmotic pressures than those actually observed might exist in the cells of the leaf.

H. H. DIXON, Dublin.

STRUCTURE OF CODIUM.—Two years ago, in the month of April, I gathered some material of *Codium tomentosum* near Ventry, Co. Kerry. In sections prepared from this for teaching purposes, I was struck by the peculiar appearance of the club-shaped ends of the ramifications of the coenocyte. Sections 10 μ thick, stained with Delafield's Haematoxylin, showed in many of the branches of the coenocyte a stout axial strand of a violet substance. The strand is thin and more delicate in the narrow interweaving parts of the coenocyte, and thickens gradually as it passes out into the enlarged terminations, which form the surface of the plant. Where it comes into contact with the protoplasmic mass occupying the extreme end of the branch of the coenocyte, it enlarges into a club- or funnel-shaped head. The strand occupies an axial position in the coenocyte and probably does not come into contact with the cell-wall at any point. In this respect it differs wholly from the transverse bars in *Caulerpa.*

In some of the branches the axial strand seems to be composed of a homogeneous refractive substance (Fig. 1). In others it is a tubular structure formed of a refractive material, lined and more or less filled up with granular matter. Where it is tubular, the ending in the protoplasm is open (Fig. 2). In the younger parts of the plant it is usually covered with a delicate coating of protoplasm, in which nuclei are sometimes seen.

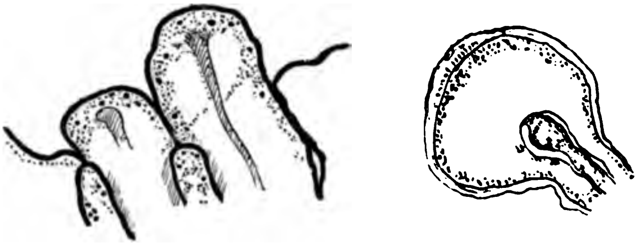

Fig 1. **Fig 2.**

Woodcut 12.

Fig. 1. Portion of a longitudinal section of *Codium tomentosum*, showing the longitudinal strands in two adjoining branches of the coenocyte. × 170.

Fig. 2. Terminal portion of a branch of the coenocyte in long section: the axial strand is tubular and open at the extremity. × 170.

In its reactions as well as in its general appearance the strand resembles some form of cellulose. It dissolves in sulphuric acid; it partially dissolves in ammoniacal cuprous oxide; it turns yellow in liquor Iodi. Neither it nor the walls of the coenocyte turned violet with chlorzinc-iodine; but it does not swell as much with this reagent as the walls, and becomes an orange yellow. The wall of the extreme end of the branches often resembles the strand in these two particulars, and differs in them from the remaining parts of the wall. Another distinction between the strand and the substance of the walls is that it often yields a dark violet colouration with liquor Iodi followed by sulphuric acid, while the walls do not. It stains readily with haematoxylin (especially the granular parts) and with methyl- or aniline-blue.

It seemed possible that this cellulose-like substance might be a reserve-material to be used up in the rapid production of the filamentous branches which occurs in this Alga during the summer. To test this point I obtained some specimens after the summer growth had taken place. For this material I am indebted to Mr. C. Green, who gathered it for me on Clare Island, last July. In it not only the branches of the coenocyte forming the compact thallus, but also those which are prolonged into the filamentous out-growths, frequently exhibit the axial strand.

It may also be interesting to note, that in this material, which was fixed in methylated spirit, one stage, at least, of nuclear division could be observed. In several instances the chromatin of the minute nuclei could be seen divided into two dome-shaped masses, the convexities being directed towards the poles of division. The surfaces of the chromatin-masses, which lay towards the equator, appeared fimbriated. The fimbriations possibly represent the ends of the chromosomes in diaster. A strand of more densely stained protoplasm connects the two masses. Except for this, I have seen no indications of a spindle. However, as only a few stains were tried, and no special care was taken in the fixing of the material, it is hardly to be expected that these details, which would be very minute in *Codium*, could be seen.

H. H. DIXON, Dublin.

ON SPENCERITES, A NEW GENUS OF LYCOPODIACEOUS CONES FROM THE COAL-MEASURES[1]. By D. H. Scott, M.A., Ph.D., F.R.S.—The fossils which form the subject of the present paper are Cryptogamic strobili, showing evident Lycopodiaceous affinities, but differing in important points from other fructifications of that family, so that it appears necessary to establish a new genus for their reception.

Two species are described, one of which (*Sbencerites insignis*) is already known to us from the investigations of Williamson, who named it first *Lepidostrobus insignis*, and afterwards *Lepidodendron Spenceri*[1], while the other (*Spencerites majusculus*) is new.

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[1] Abstract of a paper read before the Royal Society, Nov. 18, 1897.

In one of his latest publications, Williamson pointed out that it might ultimately be necessary to make his *Lepidodendron Spenceri* the type of a new genus[2]. The separation thus suggested is now carried out, on the basis of a renewed investigation of the structure of this fossil.

Spencerites insignis is a pedunculate strobilus; the vegetative organs are not as yet identified. The specimens are calcified, and their structure admirably preserved.

The anatomy of the axis is of a simple Lycopodiaceous type, but differs in details (such as the course of the leaf-trace bundles) from that of the axis of *Lepidostrobus*. The peduncle bears sterile bracts, similar to the sporophylls of the cone itself; the latter are arranged spirally, or in some cases in alternating verticils.

The individual sporophylls are of peltate form, consisting of a short cylindrical pedicel, expanding into a relatively large lamina. The sporangia are approximately spherical bodies; unlike those of *Lepidostrobus*, they are quite free from the pedicel, and are attached by a narrow base to the upper surface of the lamina, where it begins to expand.

The details of the sporangial wall are quite different from those of *Lepidostrobus*, and the spores are also characteristic. In size they are intermediate between the microspores and macrospores of *Lepidostrobus*. They are of tetrahedral form, becoming spheroidal when mature, and each spore has a hollow, annular wing running round its equator. The wing is no doubt formed by a dilation of the cuticle[3], and not, as Williamson supposed, from the abortive sister-cells.

Spencerites majusculus, the new species, is much larger than the former, the axis of the cone being twice as thick. The anatomy is similar, but the sporophylls, and consequently the leaf-traces, are more numerous. The sporophylls, which are arranged in alternating verticils, are relatively short, and of peculiar form; the lamina is very thick, and of great tangential width. The sporangia are like those of the former species, and similarly inserted, but the spores are quite different. They are smaller than those of *S. insignis*, and have the form of quadrants of a sphere, with narrow wings along their three angles.

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[1] Williamson, Organization of the Fossil Plants of the Coal-measures, Parts IX, X, XVI, and XIX, Phil. Trans., 1878, 1880, 1889, and 1893.

[2] General Index, Part II, 1893, p. 24.

[3] Cf. Solms-Laubach, Fossil Botany, p. 239.

The genus is separated from *Lepidostrobus*, mainly on account of the very different mode of insertion of the sporangia, a character which is accompanied by differences in the form of the sporophylls and sporangia, the structure of the sporangial wall and of the spores, and the whole habit of the strobilus.

Spencerites, and especially *S. insignis*, bears a considerable resemblance to the *Sigillariostrobus Crepini* of Zeiller, but cannot be united with the genus *Sigillariostrobus*, for the insertion of the sporangia in the latter, as shown in the *Sigillariostrobus ciliatus* of Kidston, is totally different. The author is much indebted both to M. Zeiller and Mr. Kidston, for the loan of their specimens for examination.

The generic and specific characters may provisionally run as follows:—

Spencerites, gen. nov.

Cone consisting of a cylindrical axis, bearing numerous simple sporophylls, arranged spirally, or in crowded alternating verticils.

Sporophylls short, formed of a sub-cylindrical pedicel, expanding into a large peltate lamina.

Sporangia solitary on each sporophyll, inserted, by a narrow base, on the upper surface of the lamina, but free from the pedicel.

Sporangial wall consisting of a single layer of prosenchymatous cells. Spores winged.

1. *Spencerites insignis* (Will.).

Lepidostrobus insignis, Will. Organization of the Fossil Plants of the Coal-measures, Part X, Phil. Trans. 1880, p. 502.

Lepidodendron Spenceri, Will. Loc. cit. Part XVI, Phil. Trans., 1889, p. 199.

Cone pedunculate; peduncle bractigerous. Whole cone 8–10 mm. in diameter. Axis 3·5–5 mm. in diameter. Sporophylls 2–2·5 mm. long; lamina distinctly peltate, vertically elongated.

Sporangia approximately spherical. Spores tetrahedral, becoming spheroidal when free, with a hollow equatorial wing. Maximum diameter of spore, without wing, about 0·14 mm.; with wing, about 0·28 mm. Wood of axis without prominent angles, with or without pith.

Outer cortex containing distinct bands of sclerenchyma.

Locality, near Halifax and Huddersfield.

Horizon, Lower Coal-measures.

2. *Spencerites majusculus*, sp. nov.

Whole cone about 15 mm. in diameter, axis about 9 mm. in diameter. Sporophylls about 3 mm. long ; lamina obscurely peltate, as seen in radial section, but greatly elongated tangentially, attaining a breadth of 3 mm.

Sporangia approximately spherical. Spores having the form of quadrants of a sphere, with three narrow wings. Maximum diameter of spore, without wings, about 0·11 mm.; with wings, about 0·15 mm.

Wood of axis with about 30, somewhat prominent, angles; without pith.

Outer cortex uniformly sclerotic.

Locality, near Halifax.

Horizon, Lower Coal-measures.

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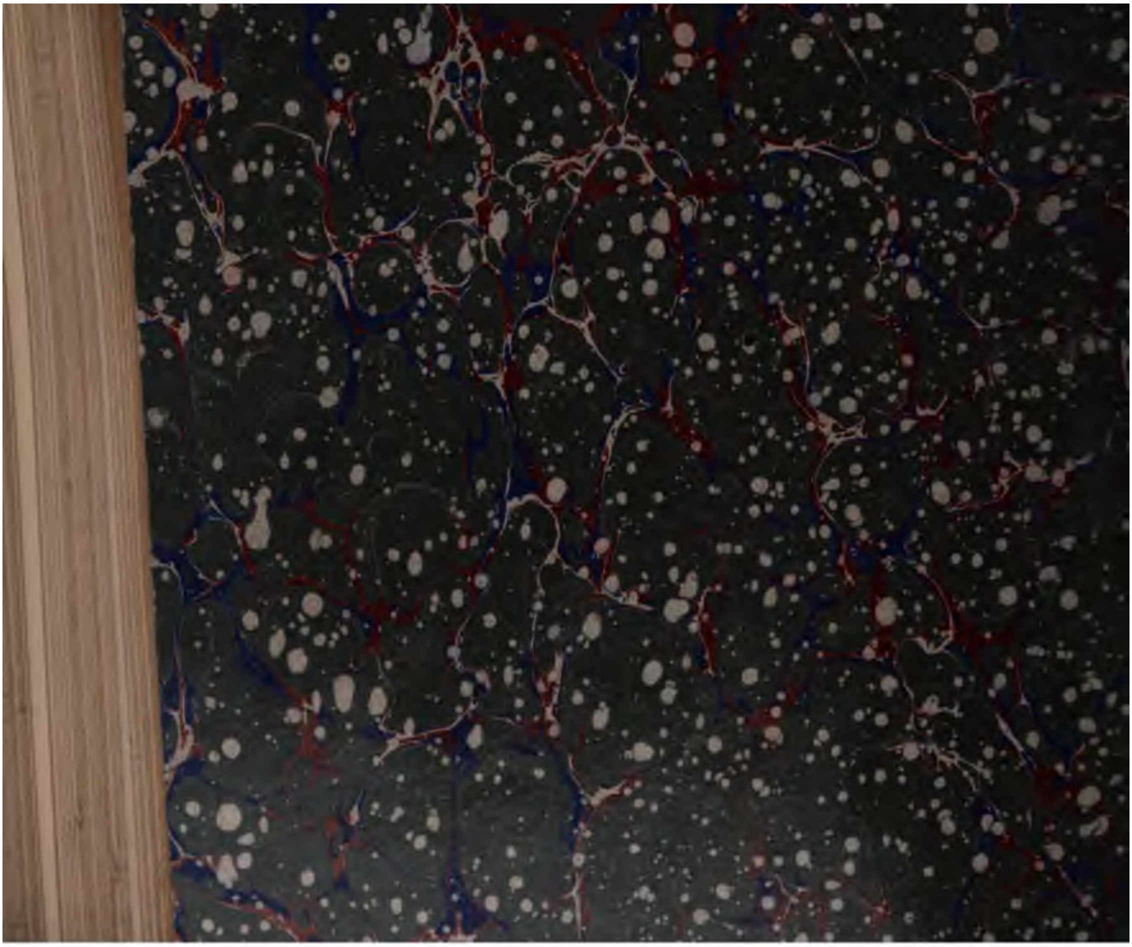

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